United States
Environmental Protection Agency

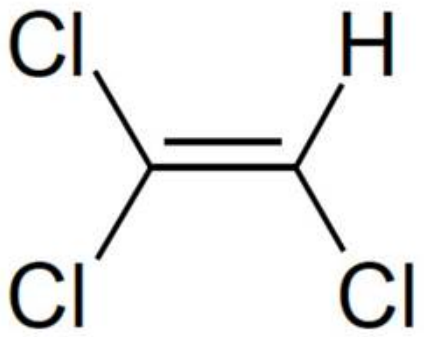
Office of Chemical Safety and
Pollution Prevention

Final Risk Evaluation for Trichloroethylene

Systematic Review Supplemental File:

Data Quality Evaluation of Human Health Hazard Studies – Animal and Mechanistic Data

CASRN: 79-01-6



November 2020

Table Listing

Acute (<24 hr)

Short-term (1-30 days)

Other

Subchronic (30-90 days)

Chronic (>90 days)

Genetic toxicity studies

Developmental and Reproductive

Mechanistic

1 Acute (<24 hr)

Table 1: **Animal toxicity evaluation results of Selgrade et al 2010 for an acute inhalation toxicity study in mice on mortality, hematological, and immune outcomes**

Study Citation:	Selgrade, MK; Gilmour, MI (2010). Suppression of pulmonary host defenses and enhanced susceptibility to respiratory bacterial infection in mice following inhalation exposure to trichloroethylene and chloroform Journal of Immunotoxicology, 7(4), 350-356					
Data Type:						
HERO ID:	730119					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified (by CASRN).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance (manufacturer) was specified. Batch/lot number was not provided, but TCE is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was > 99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Control mice were exposed to filtered air. Presumably, conditions were equal except for exposure. Mice were of the same age (5-6 weeks); body weight data were provided (no differences were reported). Body weight data was not reported, however mice were randomly assigned to treatment or filtered air groups. All groups received bacterial infection.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not indicated by study type.
	Metric 6:	Randomized Allocation	High	× 1	1	Mice were randomly assigned to treatment groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The method and equipment used to generate the test substance as a vapor were reported. Test substance storage was not reported, however this is unlikely to have a substantial impact on results, and TCE concentration was monitored within the chamber.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Exposures were administered consistently (concentrations in different areas of the chamber were within 1% of expected values). Animals were exposed for the same amount of time; the time of day was not specified. The initial number of animals/chamber was not reported. Time of day was not reported, however the Methods section implies that all mice were treated concurrently. Bacteria was aerosolized with a nebulizer. Aerosol droplet size was also not reported.

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Study Citation: Selgrade, MK; Gilmour, MI (2010). Suppression of pulmonary host defenses and enhanced susceptibility to respiratory bacterial infection in mice following inhalation exposure to trichloroethylene and chloroform Journal of Immunotoxicology, 7(4), 350-356

Data Type:

HERO ID: 730119

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	The study reported that actual concentrations monitored during exposure were within 3% of targeted concentrations; however, analytical concentrations were not reported. Chamber concentrations were monitored using long path-length dispersive infared spectrometry.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration were reported, and appears to be appropriate for this study type. This is not a common study type, but the single 3-h exposure mirrored that of a previous analogous study.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Exposure concentrations were selected based on previous studies to generate a robust concentration-response. The number of exposure groups and concentration spacing were adequate to show relevant results. For bacterial clearance from the lung, the lowest tested dose of TCE was 50ppm, despite 25ppm having a significant effect on mortality. This would not have had an effect at 72 hours but may have shown an effect at 24hrs post-exposure.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Although a dynamic chamber was used, the chamber had < 15 changes/hour (~14 changes/hour). The number of animals/chamber was not explicitly specified.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, health status (i.e., pathogen-free), and age of the test animals were reported. The lack of data on starting body weights is unlikely to have a substantial impact on results. The test animal was appropriate for the evaluation of the outcomes of interest.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Prior to exposure, animals were maintained in an environmentally controlled room on 12 hours light/dark cycles. During exposure, parameters of interest (including temperature, relative humidity, etc) were "continuously monitored" (no differences were reported),. Husbandry conditions are not likely to have a substantial impact on the results. Number of animals/cage was not reported except initially prior to group assignment and exposure.

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Study Citation: Selgrade, MK; Gilmour, MI (2010). Suppression of pulmonary host defenses and enhanced susceptibility to respiratory bacterial infection in mice following inhalation exposure to trichloroethylene and chloroform Journal of Immunotoxicology, 7(4), 350-356

Data Type:

HERO ID: 730119

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Low	× 1	3	The number of animals/group was not explicitly reported for all endpoints ("data represent a composite of three experiments with a minimum of 38 mice per exposure group"). The number of animals/group is generally sufficient for statistical analysis. Except for the mortality study, the authors' provide power analysis demonstrating that insufficient numbers of mice were used for two of the experiments, resulting in higher NOELs for what would be expected to be more sensitive endpoints.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome methodology addressed the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across exposure groups (same time after exposure and using the same protocol).
	Metric 18:	Sampling Adequacy	Medium	× 1	2	For some endpoints (percent infected mice and phagocytic index), it was indicated that sensitivity was limited by small sample size (5 animals/group).
	Metric 19:	Blinding of Assessors	Medium	× 1	2	No subjective outcomes were assessed (mortality, cell counting). This metric was determined to be acceptable and applicable (previously N/A). Most metrics were not subjective, however the number of bacteria ingested per phagocytic cell is scored somewhat subjectively. Blinding was not reported, but it is not expected to have a substantial impact.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The lack of reporting with respect to initial body weights, food/water intake, and respiratory rate is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No differences in health outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were adequately described.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were reported for all outcomes by exposure group; all data were presented graphically.

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Study Citation:	Selgrade, MK; Gilmour, MI (2010). Suppression of pulmonary host defenses and enhanced susceptibility to respiratory bacterial infection in mice following inhalation exposure to trichloroethylene and chloroform Journal of Immunotoxicology, 7(4), 350-356
Data Type:	
HERO ID:	730119

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		High		1.6	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 2: **Animal toxicity evaluation results of Kim et al 2011 after a single dose oral exposure study in rats for gene expression/omics outcomes**

Study Citation:	Kim, J.K., Jung, K.H., Noh, J.H., Eun, J.W., Bae, H.J., Xie, H.J., Jang, J.J. Ryu, J.C., Park, W.S., Lee, J.Y., Nam, S.W. (2011). Identification of characteristic molecular signature for volatile organic compounds in peripheral blood of rat Toxicology and Applied Pharmacology, 250(2), 162-169
Data Type:	Study of gene expression changes in rats after single dose oral exposure, with limited toxicity data (omics)
HERO ID:	1788091

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name and CASRN
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance source was reported but without batch/lot number or certification or analytical verification of identity.
	Metric 3:	Test Substance Purity	Low	× 1	3	Neither purity nor grade were reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham-treated controls were given vehicle.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not typical for this type of study.
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation and storage methods were not reported; preparation method was cited to the manufacturer. However, the lack of storage information is not a major limitation as it is a single dose study.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	No information on gavage volume or time of day of administration was reported.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Unambiguous doses were reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Frequency and duration were reported and suited to the study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	2 dose groups were selected based on fractions of the LD50.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The method of administration was not reported, but is reasonably presumed to be gavage given the nature of the exposure levels reported (mg/kg) and vehicle (corn oil)
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Test animal source was not reported
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most animal husbandry conditions were reported and customary, but housing (cages and numbers per cage) was not described.

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Study Citation:	Kim, J.K., Jung, K.H., Noh, J.H., Eun, J.W., Bae, H.J., Xie, H.J., Jang, J.J. Ryu, J.C., Park, W.S., Lee, J.Y., Nam, S.W. (2011). Identification of characteristic molecular signature for volatile organic compounds in peripheral blood of rat Toxicology and Applied Pharmacology, 250(2), 162-169
Data Type:	Study of gene expression changes in rats after single dose oral exposure, with limited toxicity data (omics)
HERO ID:	1788091

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	Exposed groups consisted of 7/dose with 12 controls; size of exposed groups is somewhat small.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported and sensitive for the endpoint (whole genome microarray with QRT-PCR validation)
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No inconsistencies in outcome assessment were reported, and adequate information on assessment was provided.
	Metric 18:	Sampling Adequacy	High	× 1	1	Endpoints were evaluated in all animals.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Endpoints were not subjective
	Metric 20:	Negative Control Response	High	× 1	1	No concerns with the control response were apparent
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No information on potential confounding variables was presented.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	No information on attrition or health outcomes other than the measured endpoints was reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was reported and appropriate for the endpoints.
	Metric 24:	Reporting of Data	High	× 2	2	Response data were reported graphically as is typical for omics data.
Overall Quality Determination‡			Medium		1.8	
Extracted			No			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 3: **Animal toxicity evaluation results of Kim et al 2011 after a single dose oral exposure study in rats for hepatic, renal, and respiratory outcomes**

Study Citation:	Kim, J.K., Jung, K.H., Noh, J.H., Eun, J.W., Bae, H.J., Xie, H.J., Jang, J.J. Ryu, J.C., Park, W.S., Lee, J.Y., Nam, S.W. (2011). Identification of characteristic molecular signature for volatile organic compounds in peripheral blood of rat Toxicology and Applied Pharmacology, 250(2), 162-169
Data Type:	Study of gene expression changes in rats after single dose oral exposure, with limited toxicity data
HERO ID:	1788091

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name and CASRN
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance source was reported but without batch/lot number or certification or analytical verification of identity.
	Metric 3:	Test Substance Purity	Low	× 1	3	Neither purity nor grade were reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham-treated controls were given vehicle.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not typical for this type of study.
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation and storage methods were not reported; preparation method was cited to the manufacturer. However, the lack of storage information is not a major limitation as it is a single dose study.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	No information on gavage volume or time of day of administration was reported.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Unambiguous doses were reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Frequency and duration were reported and suited to the study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	2 dose groups were selected based on fractions of the LD50.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The method of administration was not reported, but is reasonably presumed to be gavage given the nature of the exposure levels reported (mg/kg) and vehicle (corn oil)
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Test animal source was not reported
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most animal husbandry conditions were reported and customary, but housing (cages and numbers per cage) was not described.

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Study Citation:	Kim, J.K., Jung, K.H., Noh, J.H., Eun, J.W., Bae, H.J., Xie, H.J., Jang, J.J. Ryu, J.C., Park, W.S., Lee, J.Y., Nam, S.W. (2011). Identification of characteristic molecular signature for volatile organic compounds in peripheral blood of rat Toxicology and Applied Pharmacology, 250(2), 162-169
Data Type:	Study of gene expression changes in rats after single dose oral exposure, with limited toxicity data
HERO ID:	1788091

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	Exposed groups consisted of 7/dose with 12 controls; size of exposed groups is somewhat small.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported and sensitive for the endpoint (organ weight and histopathology for liver, kidney, and lung; some clinical chemistry).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No inconsistencies in outcome assessment were reported, and adequate information on assessment was provided.
	Metric 18:	Sampling Adequacy	High	× 1	1	All endpoints were evaluated in all animals.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not typical for initial histopathology review; remaining endpoints were not subjective.
	Metric 20:	Negative Control Response	Low	× 1	3	Control response data were not reported but are available in a supplemental file.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No information on potential confounding variables was presented.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	No information on attrition or health outcomes other than the measured endpoints was reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical analysis was not reported, and data for independent analysis were not reported but are available in a supplemental file.
	Metric 24:	Reporting of Data	Low	× 2	6	Response data were not reported quantitatively but are available in a supplemental file.
Overall Quality Determination‡			Medium		2.0	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 4: **Animal toxicity evaluation results of Yaqoob et al 2013 for an acute toxicity study in rats on nutrition and metabolic/adult exposure body weight, hepatic, and renal outcomes**

Study Citation:	Yaqoob, N., Evans, A.R., Lock, E.A. (2013). Trichloroethylene-induced formic aciduria: Effect of dose, sex and strain of rat Toxicology, 304 49-56					
Data Type:	Acute toxicity					
HERO ID:	1790783					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported, including manufacturer and grade.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was acceptable (reported to be 98% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative control group (received the vehicle, corn oil) was used and was appropriate.
	Metric 5:	Positive Controls	Not Rated	NA	NA	N/A - Positive control is not indicated by the study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study authors did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation and storage conditions were reported and were appropriate for the test substance.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups in a scientifically sound manner (dose volume 5 mL/kg/day was acceptable).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The administered doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	There were minor limitations regarding the number of exposure groups in some tests (i.e., only one quantitative TCE dose of 1000 mg/kg/day was administered to male rats in a 3-day TCE exposure test).
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and suited to the test substance.
Domain 4: Test Organism						

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Study Citation:	Yaqoob, N., Evans, A.R., Lock, E.A. (2013). Trichloroethylene-induced formic aciduria: Effect of dose, sex and strain of rat Toxicology, 304 49-56
Data Type:	Acute toxicity
HERO ID:	1790783

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, age, and starting body weight were reported; however, downgraded to medium because health status was not reported,. The animals were obtained from a laboratory-maintained colony.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions, including ranges for temperature and humidity, and light-dark cycle duration, were reported and were adequate and the same for the control and exposed populations.
	Metric 15:	Number per Group	Medium	× 1	2	The reported number of animals per study group (3 in some tests) was lower than the typical number used in studies of the same or similar type but was sufficient for statistical analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcomes (primarily evaluating formic aciduria but reported mortality, body weight, organ weight outcomes and urinary acidification) of interest and was sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	The outcome assessment methodology was not reported (e.g., no details were reported on when body weights or urine volume were measured).
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding sampling of outcomes were not reported and this deficiency is likely to have a substantial impact on results.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were reported that required blinding.
	Metric 20:	Negative Control Response	Medium	× 1	2	The biological responses of the control group were reported for some, but not all, reported outcomes.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weights, food/water intake, and respiratory rate were not reported but lack of reporting is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted by the study authors.
Domain 7: Data Presentation and Analysis						

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Study Citation:	Yaqoob, N., Evans, A.R., Lock, E.A. (2013). Trichloroethylene-induced formic aciduria: Effect of dose, sex and strain of rat Toxicology, 304 49-56
Data Type:	Acute toxicity
HERO ID:	1790783

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 23: Statistical Methods	High	× 1	1	Statistical methods were described only partially (e.g., ANOVA was reported but post-hoc tests, if used, were not clearly described for some analyses, such as urinary acidification in Figure 2). However, data were provided that would allow an independent evaluation of the exposure-related effects.
	Metric 24: Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes and were acceptable.
Overall Quality Determination‡		High		1.5	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 5: **Animal toxicity evaluation results of Fang et al 2013 for an acute gavage study on hepatic, ADME/PBPK, and gene expression/omics outcomes**

Study Citation:	Fang, Z.Z., Krausz, K.W., Tanaka, N., Li, F., Qu, A., Idle, J.R., Gonzales, F.J. (2013). Metabolomics reveals trichloroacetate as a major contributor to trichloroethylene-induced metabolic alterations in mouse urine and serum Archives of Toxicology, 87(11), 1975-1987					
Data Type:	Acute gavage study					
HERO ID:	2127961					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name.
	Metric 2:	Test Substance Source	Medium	× 1	2	Manufacturer was identified, but no lot no. No analytical verification.
	Metric 3:	Test Substance Purity	High	× 1	1	>=99.5% purity
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Corn oil vehicle controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not used for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not report how animals were allocated to study group.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was described and stability over the test period was noted.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups. Provided additional references for the details on exposure methods
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Gavage doses reported by the study authors.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single gavage dose was adequate for the measured outcomes. CK: Mice were given TCE at 800 or 1,600 mg/kg body weight per day by oral gavage as previously described (Griffin et al. 2000; Ramdhan et al. 2010)
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Doses justified based on previous studies.
	Metric 12:	Exposure Route and Method	High	× 1	1	Corn oil is an appropriate oral vehicle for TCE.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain, sex and age were provided,

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Study Citation: Fang, Z.Z., Krausz, K.W., Tanaka, N., Li, F., Qu, A., Idle, J.R., Gonzales, F.J. (2013). Metabolomics reveals trichloroacetate as a major contributor to trichloroethylene-induced metabolic alterations in mouse urine and serum Archives of Toxicology, 87(11), 1975-1987
Data Type: Acute gavage study
HERO ID: 2127961

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions were not adequately reported. CK: But followed the reference guidance: All animal experiments were conducted in accordance with animal study protocols approved by the National Cancer Institute Animal Care and Use Committee under Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines.
	Metric 15:	Number per Group	High	× 1	1	5/group; adequate for acute experiment.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Liver weight and ALT/AST, but no histopathology. CK: Histopathological analysis using hematoxylin and eosin staining of liver sections (Supplemental Figs. 5, 6) further showed hepatocyte hypertrophy after treatment with TCE, DCA, and TCA. Very mild focal inflammation was detected in TCE-treated mice (Supplemental Fig. 5). However, obvious steatosis, hepatocyte necrosis, and cholestasis were not observed. (on page 1978- under results)
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	CK: Adequate information on assessment was provided.
	Metric 18:	Sampling Adequacy	High	× 1	1	CK: Endpoints were evaluated in all animals.
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported; however, the outcomes were subjective. CK: however, the outcomes were NOT subjective.
	Metric 20:	Negative Control Response	High	× 1	1	CK: No concerns with the control response were apparent
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No difference in initial bw and food intake among groups (Supplemental file Fig. 1)
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	CK: Statistical analysis was reported and appropriate for the endpoints.

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Study Citation:	Fang, Z.Z., Krausz, K.W., Tanaka, N., Li, F., Qu, A., Idle, J.R., Gonzales, F.J. (2013). Metabolomics reveals trichloroacetate as a major contributor to trichloroethylene-induced metabolic alterations in mouse urine and serum Archives of Toxicology, 87(11), 1975-1987
Data Type:	Acute gavage study
HERO ID:	2127961

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	Medium	× 2	4	ALT/AST data were reported in supplemental file.
Overall Quality Determination‡		High		1.4	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 6: **Animal toxicity evaluation results of Kim et al 2013 for a single oral gavage exposure study in rats on gene expression/omics outcomes**

Study Citation:	Kim, JK; Eun, JW; Bae, HJ; Shen, Q; Park, SJ; Kim, HS; Park, S; Ahn, YM; Park, WS; Lee, JY; Nam, SW (2013). Characteristic molecular signatures of early exposure to volatile organic compounds in rat liver Biomarkers, 18(8), 706-715					
Data Type:	Single oral gavage exposure in rats (omics study)					
HERO ID:	2800143					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name and CASRN
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance source identified without certification or analytical verification of identity.
	Metric 3:	Test Substance Purity	Low	× 1	3	Neither purity nor grade was reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative vehicle controls were referenced but their treatment was not described.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for this study type
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not report method of allocation
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Study reports that test materials were prepared following the manufacturer's protocol, but storage conditions were not reported
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Study did not report time of day of gavage administration. TCE absorption is influenced by fasting.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported in mg/kg bw.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration reported and suited to study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Two nonzero doses differing by 5-fold were used (LD50 and LD10). The administered doses resulted in gene expression changes.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Gavage volume was not reported.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Test animal source was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most husbandry conditions were reported and adequate; housing was not described (cage type, number per cage). No inconsistencies in husbandry conditions were reported.

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Study Citation:	Kim, JK; Eun, JW; Bae, HJ; Shen, Q; Park, SJ; Kim, HS; Park, S; Ahn, YM; Park, WS; Lee, JY; Nam, SW (2013). Characteristic molecular signatures of early exposure to volatile organic compounds in rat liver Biomarkers, 18(8), 706-715
Data Type:	Single oral gavage exposure in rats (omics study)
HERO ID:	2800143

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Low	× 1	3	Number of animals/group is reported both as 7 "administered at a high- and low- dose to seven male" and 14 "Fourteen animals were allocated to each treatment group". There were 12 controls however, only 9 were used in the microarray study due to poor quality RNA .
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Microarray analysis was described in detail but expression changes were not confirmed by RT-PCR
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No inconsistencies in outcome assessment were reported, except that RNA quality was poor in some controls, affecting the sampling adequacy.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Microarray analysis in controls could be performed only for 9/12 animals due to poor quality RNA in the remaining 3.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Outcomes were not subjective
	Metric 20:	Negative Control Response	High	× 1	1	No issues with control response were noted apart from those affecting sampling adequacy.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Study does not report initial body weights . This study was one exposure of two different concentrations in which endpoints were assessed 48 hrs after exposure, food and water intake would have little to no impact on the outcome. No potential confounding factors were noted.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	No concerns with other health outcomes were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis of omics outcomes was conducted, described in detail, and appeared appropriate to the endpoint.
	Metric 24:	Reporting of Data	High	× 2	2	Reporting of omics data was consistent with usual methods. Additional details are available in a supplemental file.
Overall Quality Determination‡			Medium		1.9	
Extracted			No			

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Study Citation:	Kim, JK; Eun, JW; Bae, HJ; Shen, Q; Park, SJ; Kim, HS; Park, S; Ahn, YM; Park, WS; Lee, JY; Nam, SW (2013). Characteristic molecular signatures of early exposure to volatile organic compounds in rat liver Biomarkers, 18(8), 706-715
Data Type:	Single oral gavage exposure in rats (omics study)
HERO ID:	2800143

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 7: **Animal toxicity evaluation results of Kim et al 2013 for a single oral gavage exposure study in rats on body and organ weight and clinical chemistry outcomes**

Study Citation:	Kim, JK; Eun, JW; Bae, HJ; Shen, Q; Park, SJ; Kim, HS; Park, S; Ahn, YM; Park, WS; Lee, JY; Nam, SW (2013). Characteristic molecular signatures of early exposure to volatile organic compounds in rat liver Biomarkers, 18(8), 706-715
Data Type:	Single oral gavage exposure in rats at two different concentrations
HERO ID:	2800143

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name and CASRN
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance source identified without certification or analytical verification of identity.
	Metric 3:	Test Substance Purity	Low	× 1	3	Neither purity nor grade was reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative vehicle controls were referenced but their treatment was not described.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for this study type
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not report method of allocation
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Study reports only that test materials were prepared following the manufacturer's protocol but storage information was not provided
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Study did not report time of day of gavage administration. TCE absorption is influenced by fasting.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported in mg/kg bw.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration reported and suited to study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Two nonzero doses differing by 5-fold were used. The high dose did not alter any apical endpoints so it is not clear that the dose was high enough.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Gavage volume was not reported.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Test animal source was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most husbandry conditions were reported and adequate; housing was not described (cage type, number per cage). No inconsistencies in husbandry conditions were reported.
	Metric 15:	Number per Group	Low	× 1	3	Number of animals/group is reported both as 7 "administered at a high- and low- dose to seven male" and 14 "Fourteen animals were allocated to each treatment group". There were 12 controls.

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Study Citation:	Kim, JK; Eun, JW; Bae, HJ; Shen, Q; Park, SJ; Kim, HS; Park, S; Ahn, YM; Park, WS; Lee, JY; Nam, SW (2013). Characteristic molecular signatures of early exposure to volatile organic compounds in rat liver Biomarkers, 18(8), 706-715
Data Type:	Single oral gavage exposure in rats at two different concentrations
HERO ID:	2800143

Domain	Metric		Rating†	MWF*	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Study evaluated few and generally insensitive apical endpoints (hepatic serum enzymes and body, liver, kidney, and lung weights), and cites "standard" methods for serum chemistry. Primary focus was omics. NK: Before and after body weights were not reported.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No inconsistencies in outcome assessment execution were reported.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Study did not report outcome sampling size for endpoints other than omics.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Outcomes were not subjective
	Metric 20:	Negative Control Response	Low	× 1	3	Control response was not reported quantitatively so it is not possible to assess.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Study does not report initial or final body weights . Because it is a single dose study (at two different concentrations) in which endpoints were assessed 48 hrs after exposure, food and water intake would have little to no impact on the outcome. No potential confounding factors were noted.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	No concerns with other health outcomes were noted..
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	Statistical analysis of toxicity endpoints was not conducted or reported, and data enabling independent analysis were not reported.
	Metric 24:	Reporting of Data	Low	× 2	6	Toxicity data were reported qualitatively with little detail. NK:The only information reported is ".....exposure to high or low doses of VOCs did not affect body and organ (liver, lung, and kidney) weights at 48 h post-administration as compared with vehicle-treated normal controls (controls)".
Overall Quality Determination‡			Unacceptable**		2.3	
Extracted			No			

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Study Citation:	Kim, JK; Eun, JW; Bae, HJ; Shen, Q; Park, SJ; Kim, HS; Park, S; Ahn, YM; Park, WS; Lee, JY; Nam, SW (2013). Characteristic molecular signatures of early exposure to volatile organic compounds in rat liver Biomarkers, 18(8), 706-715
Data Type:	Single oral gavage exposure in rats at two different concentrations
HERO ID:	2800143

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 8: **Animal toxicity evaluation results of ICI Americas 1991 for a 6-hr inhalation study in mice on respiratory and ADME/PBPK outcomes**

Study Citation:	ICI Americas Inc (1991). Initial submission: A mechanism for the development of Clara cell lesions in the mouse lung after exposure to trichloroethylene with cover letter dated 072391					
Data Type:	Animal toxicity evaluation results of ICI Americas 1991 for a 6-hr inhalation toxicity study on respiratory and ADME/PBPK outcomes					
HERO ID:	4215741					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance including the manufacturer was reported, but the batch/lot number was not reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity was not provided, but was reported to be Aristar grade which exceeds ACS grade, Therefore, the effects are likely due to the test substance. The purity of radioactive (14C-labeled) TCE was reported to be >98%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control animals were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not required.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Deficiencies in reporting of test substance preparation and/or storage conditions are likely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups in a scientifically sound manner.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Study reported method for vapor generation of TCE and the actual concentrations, but have not reported the target concentration. It is reported that the chamber atmospheric concentrations of TCE were monitored but the results were not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and/or outcome(s) of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of groups and spacing were reported and sufficient to show results relevant to the outcome of interest.

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Study Citation:	ICI Americas Inc (1991). Initial submission: A mechanism for the development of Clara cell lesions in the mouse lung after exposure to trichloroethylene with cover letter dated 072391
Data Type:	Animal toxicity evaluation results of ICI Americas 1991 for a 6-hr inhalation toxicity study on respiratory and ADME/PBPK outcomes
HERO ID:	4215741

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The source, species, strain, sex, initial body weight was reported. The health status was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions were not sufficiently reported - temperature and light/dark cycle were reported, but specifics were missing. Humidity was not reported.
	Metric 15:	Number per Group	Low	× 1	3	The study did not explicitly reported the number of animals dosed in the methods section. However, there is a mention of the number of animals used in the different assays ranging from 3-6 and most of them are used as pooled. This number is lower than required for a subchronic study and also since the tissues are sometimes pooled.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	There were deficiencies in the reported outcome assessment methodologies. This is likely to have a substantial impact on results.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There were incomplete reporting of minor details of outcome assessment protocol execution, but these uncertainties or limitations are unlikely to have substantial impact on results.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcome(s) of interest were reported and adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not applicable.
	Metric 20:	Negative Control Response	High	× 1	1	Negative controls responded appropriately.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Study did not report initial body weight, food/water intake, and respiratory rate. These deficiencies are likely to have a substantial impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical analysis was not described clearly, and this deficiency is likely to have a substantial impact on results.

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Study Citation:	ICI Americas Inc (1991). Initial submission: A mechanism for the development of Clara cell lesions in the mouse lung after exposure to trichloroethylene with cover letter dated 072391
Data Type:	Animal toxicity evaluation results of ICI Americas 1991 for a 6-hr inhalation toxicity study on respiratory and ADME/PBPK outcomes
HERO ID:	4215741

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	Medium	× 2	4	Data were reported for some, but not all, outcomes by exposure group.
Overall Quality Determination‡		Medium		2.0	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 9: **Animal toxicity evaluation results of NCI 1976 for a single dose gavage in rats study on mortality outcomes**

Study Citation:	NCI (1976). Carcinogenesis bioassay of trichloroethylene					
Data Type:	Single dose gavage study in rats					
HERO ID:	75178					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test material identified by unambiguous name
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained from manufacturer and identified by lot number and date of receipt
	Metric 3:	Test Substance Purity	High	× 1	1	Purity verified by GC and infrared spectroscopy to be at least 99%; impurities identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	A negative control group was not reported for this acute lethality study.
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	High	× 1	1	Study reports that the animals were allocated randomly (albeit not by random number table) such that initial average weight of each group was approximately equal.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Details of preparation and storage were reported and should have been adequate to preserve TCE stability (e.g., sealed and refrigerated containers), but stability was not tested.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Little information was provided on consistency of exposure administration in the acute lethality study.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Gavage volumes were not reported for the acute lethality study.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single dose exposure is typical for acute lethality studies.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Ten nonzero doses were administered, with a range over 178-fold. Dose range and spacing was sufficient to identify effect and no-effect levels.
	Metric 12:	Exposure Route and Method	High	× 1	1	Gavage administration was reported and appropriate to the study type.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animals were appropriate and obtained from commercial source, with strain, age, sex, and BW, but age at initiation of the acute lethality study was not reported. In addition, only male rats were tested.

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Study Citation: NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type: Single dose gavage study in rats
HERO ID: 75178

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were reported in detail and no deviations were reported. The animals were housed in the same room with animals used in other experiments, but this is not expected to affect the results because both control and exposed groups were housed in the same room.
	Metric 15:	Number per Group	Medium	× 1	2	The acute lethality study used group sizes of 2/dose; this group size is smaller than the number recommended by OECD (3/dose) but higher than the number used in the up and down method (1/dose)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Mortality within 14 days was the only outcome assessed; clinical signs and body weights were not evaluated.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	There were no reported inconsistencies in outcome assessment across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Mortality does not require sampling
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Death is not subjective
	Metric 20:	Negative Control Response	Not Rated	NA	NA	There were no negative controls
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	No variations in test execution were reported, but few details were provided for the acute lethality study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Not Rated	NA	NA	Only mortality was evaluated.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	Statistical analysis was not performed and data enabling independent analysis were not reported.
	Metric 24:	Reporting of Data	Unacceptable	× 2	8	Only the lowest dose resulting in death was reported.
Overall Quality Determination‡			Unacceptable⋆⋆		2.2	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 10: **Animal toxicity evaluation results of NCI 1976 for a single dose gavage in mice study on mortality outcomes**

Study Citation:	NCI (1976). Carcinogenesis bioassay of trichloroethylene					
Data Type:	Single dose gavage study in mice					
HERO ID:	75178					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test material identified by unambiguous name
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained from manufacturer and identified by lot number and date of receipt
	Metric 3:	Test Substance Purity	High	× 1	1	Purity verified by GC and infrared spectroscopy to be at least 99%; impurities identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	A negative control group was not reported for this acute lethality study.
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	High	× 1	1	Study reports that the animals were allocated randomly (albeit not by random number table) such that initial average weight of each group was approximately equal.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Details of preparation and storage were reported and should have been adequate to preserve TCE stability (e.g., sealed and refrigerated containers), but stability was not tested.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Little information was provided on consistency of exposure administration in the acute lethality study.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Gavage volumes were not reported for the acute lethality study.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single dose exposure is typical for acute lethality studies.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Ten nonzero doses were administered, with a range over 178-fold. Dose range and spacing was sufficient to identify effect and no-effect levels.
	Metric 12:	Exposure Route and Method	High	× 1	1	Gavage administration was reported and appropriate to the study type.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animals were appropriate and obtained from commercial source, with strain, age, sex, and BW, but age at initiation of the acute lethality study was not reported. In addition, only female mice were tested.

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Study Citation: NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type: Single dose gavage study in mice
HERO ID: 75178

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were reported in detail and no deviations were reported. The animals were housed in the same room with animals used in other experiments, but this is not expected to affect the results because both control and exposed groups were housed in the same room.
	Metric 15:	Number per Group	Medium	× 1	2	The acute lethality study used group sizes of 2/dose; this group size is smaller than the number recommended by OECD (3/dose) but higher than the number used in the up and down method (1/dose)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Mortality within 14 days was the only outcome assessed; clinical signs and body weights were not evaluated.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	There were no reported inconsistencies in outcome assessment across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Mortality does not require sampling
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Death is not subjective
	Metric 20:	Negative Control Response	Not Rated	NA	NA	There were no negative controls
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	No variations in test execution were reported, but few details were provided for the acute lethality study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Not Rated	NA	NA	Only mortality was evaluated.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	Statistical analysis was not performed and data enabling independent analysis were not reported.
	Metric 24:	Reporting of Data	Unacceptable	× 2	8	Only the lowest dose resulting in death was reported.
Overall Quality Determination‡			Unacceptable⋆⋆		2.2	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 11: **Animal toxicity evaluation results of Wahlberg and Boman 1979 for an acute percutaneous toxicity in guinea pig**

Study Citation:	Wahlberg, JE; Boman, A (1979). Comparative percutaneous toxicity of ten industrial solvents in the guinea pig Scandinavian Journal of Work, Environment and Health, 5(4,4), 345-351
Data Type:	acute percutaneous toxicity in guinea pig
HERO ID:	61688

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substances were identified; however, the test substances were lacking characterization details; unlikely to have a substantial impact on results.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substances were identified; did not include batch/lot numbers; unlikely to have a substantial impact on results.
	Metric 3:	Test Substance Purity	Low	× 1	3	purity or grade of test substances were not reported; possible impurities were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Distilled water was used as a concurrent control
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not rated/applicable; positive control was not indicated by study type
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	There were no details of test substance preparation and/or storage conditions reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Single application to skin depot (31 cm2) and covered CK: Not 31 cm2. The solvents was administered to a skin depot area 3.1 cm2
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	applied concentrations were reported in ml; mean body weight was reported to estimate an administered dose.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	single application, covered, and observed for 35 d
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups for CCl4 = 2 and TCE=1; number of exposure groups and spacing were not justified by the author; Doses were considered adequate to address the purpose of the study for changes in body weight for both CCl4 and TCE; however for TCE, it is unclear if the exposure level was adequate to show results relevant to mortality as there were no effects at the single concentration tested.

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Study Citation:	Wahlberg, JE; Boman, A (1979). Comparative percutaneous toxicity of ten industrial solvents in the guinea pig Scandinavian Journal of Work, Environment and Health, 5(4,4), 345-351
Data Type:	acute percutaneous toxicity in guinea pig
HERO ID:	61688

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substances
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source, strain, or sex of the test guinea pigs were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate
	Metric 15:	Number per Group	High	× 1	1	20 animals per series
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcomes of interest and was sensitive for the outcomes of interest; mortality was monitored and body weight was recorded
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	High	× 1	1	Mortality observations and weight measurements were made for all animals daily except weekends
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	this metric is not rated/applicable because no subjective outcomes were assessed.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group(s) were adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weights were reported; there was no reporting of food/water intake; unlikely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Noted that an analysis of variance was applied in the statistical calculations, though statistical tests were not specified. P-values (unspecified significance test) were reported for body weight changes. No statistical significance values were reported for mortality
	Metric 24:	Reporting of Data	Medium	× 2	4	Incidence of mortality was reported for both CCl4 and TCE. Body weight changes was reported for TCE, but not CCl4

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Study Citation:	Wahlberg, JE; Boman, A (1979). Comparative percutaneous toxicity of ten industrial solvents in the guinea pig Scandinavian Journal of Work, Environment and Health, 5(4,4), 345-351
Data Type:	acute percutaneous toxicity in guinea pig
HERO ID:	61688

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		Medium		1.9	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

2 Short-term (1-30 days)

Table 12: **Animal toxicity evaluation results of Woolhiser et al 2006 for a 4-wk inhalation immunotoxicity study in rats on hematological, immune, and respiratory outcomes**

Study Citation:	Woolhiser, MR; Krieger, SM; Thomas, J; Hotchkiss, JA (2006). Trichloroethylene (TCE): Immunotoxicity potential in CD rats following a 4-week vapor inhalation exposure
Data Type:	4-week inhalation immunotoxicity study
HERO ID:	730431

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name, synonyms and structure.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source and lot no. were reported.
	Metric 3:	Test Substance Purity	High	× 1	1	>99.9% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Air controls
	Metric 5:	Positive Controls	High	× 1	1	Cyclophosphamide was used as a positive control (data reported for all endpoints).
	Metric 6:	Randomized Allocation	High	× 1	1	Randomly assigned using a computer program.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Method and equipment used to generate vapor were will described.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure details were well-reported.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Nominal and measured concentrations were reported (IR spectophotometer measurements).. Deviation from targeted values was <10%.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	6 h/day, 5 days/week for 4 weeks
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	3 concentrations plus controls; spacing appears adequate.
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic ehole-body chamber; 12-15 air exchanges/hour; unclear about whether condensation would occur under study consitions.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Commercial source, species, strain, age and health status (virus antibody free) were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were well-reported and adequate.
	Metric 15:	Number per Group	Medium	× 1	2	8 females/group
Domain 5: Outcome Assessment						

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Study Citation:	Woolhiser, MR; Krieger, SM; Thomas, J; Hotchkiss, JA (2006). Trichloroethylene (TCE): Immunotoxicity potential in CD rats following a 4-week vapor inhalation exposure
Data Type:	4-week inhalation immunotoxicity study
HERO ID:	730431

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Assay of immune function in addition to BAL analysis, clinical signs, histopathology, organ wt. etc.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported, but outcomes were not subjective.
	Metric 20:	Negative Control Response	High	× 1	1	Histopathology was reported as within normal limits for controls.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate is not measured; TEC is expected to be an respiratory irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	
	Metric 24:	Reporting of Data	High	× 2	2	Data tables were reported for all outcomes.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 13: **Animal toxicity evaluation results of Woolhiser et al 2006 for a 4-wk inhalation study in rats on hepatic and renal outcomes**

Study Citation:	Woolhiser, MR; Krieger, SM; Thomas, J; Hotchkiss, JA (2006). Trichloroethylene (TCE): Immunotoxicity potential in CD rats following a 4-week vapor inhalation exposure
Data Type:	4-week inhalation study
HERO ID:	730431

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name, synonyms and structure.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source and lot no. were reported.
	Metric 3:	Test Substance Purity	High	× 1	1	>99.9% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Air controls
	Metric 5:	Positive Controls	High	× 1	1	Cyclophosphamide was used as a positive control (data reported for all endpoints).
	Metric 6:	Randomized Allocation	High	× 1	1	Randomly assigned using a computer program.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Method and equipment used to generate vapor were will described.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure details were well-reported.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Nominal and measured concentrations were reported (IR spectophotometer measurements).. Deviation from targeted values was <10%.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	6 h/day, 5 days/week for 4 weeks
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	3 concentrations plus controls; spacing appears adequate.
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic ehole-body chamber; 12-15 air exchanges/hour; unclear about whether condensation would occur under study consitions.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Commercial source, species, strain, age and health status (virus antibody free) were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were well-reported and adequate.
	Metric 15:	Number per Group	Medium	× 1	2	8 females/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Only organ wt. and gross pathology findings were evaluated for liver and kidney; no clinical chemistry, urinalysis or histopathology.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	

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Study Citation:	Woolhiser, MR; Krieger, SM; Thomas, J; Hotchkiss, JA (2006). Trichloroethylene (TCE): Immunotoxicity potential in CD rats following a 4-week vapor inhalation exposure
Data Type:	4-week inhalation study
HERO ID:	730431

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported, but outcomes were not subjective.
	Metric 20:	Negative Control Response	High	× 1	1	Histopathology was reported as within normal limits for controls.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate is not measured; TEC is expected to be an respiratory irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	
	Metric 24:	Reporting of Data	High	× 2	2	Data tables were reported for all outcomes.
Overall Quality Determination‡			~~High~~ ⟶ Medium§		~~1.3~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "No clinical chemistry, urinalysis or histopathology evaluations were performed."

Table 14: **Animal toxicity evaluation results of Kobayashi et al 2010 for an immunotoxicity screen in ova-sensitized mice for 2 or 4 wks on hematological, immune, and body weight outcomes**

Study Citation:	Kobayashi, R., Ikemoto, T., Seo, M., Satoh, M., Inagaki, N., Nagai, H., Nagase, H. (2010). Enhancement of immediate allergic reactions by trichloroethylene ingestion via drinking water in mice Journal of Toxicological Sciences, 35(5), 699-707					
Data Type:	Immunotoxicity Screen in OVA-sensitized mice					
HERO ID:	1101921					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE
	Metric 2:	Test Substance Source	Medium	× 1	2	Nacalai Tesqu, Inc.; lot/batch not reported CK: purchased from Nacalai Tesque, Inc. (Kyoto, Japan)
	Metric 3:	Test Substance Purity	Low	× 1	3	purity not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls, with or without OVA injections (for sensitization)
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not required by study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	300 mg/L TCE stock solution was prepared in DMSO. Diluted with distilled water to desired concentrations for use in drinking water.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Water was changed every other day to ensure dose maintenance and water intake was monitored. No separate analysis of concentrations in drinking water solutions.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Report target doses and analytical doses calculated based on measured water intake and body weight.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	2 or 4 weeks (drinking water available ad libitum)
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	2 exposure levels, plus controls. Half of each group injected with OVA (for sensitization), half unexposed to OVA.
	Metric 12:	Exposure Route and Method	High	× 1	1	drinking water, changed every other day
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Male BALB/c mice (Japan SLC), 7-9 wk old at study initiation. Initial BW not reported. Quarantined for a week. Half of the animals in each group were sensitized with OVA, other half were not.

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Study Citation:	Kobayashi, R., Ikemoto, T., Seo, M., Satoh, M., Inagaki, N., Nagai, H., Nagase, H. (2010). Enhancement of immediate allergic reactions by trichloroethylene ingestion via drinking water in mice Journal of Toxicological Sciences, 35(5), 699-707
Data Type:	Immunotoxicity Screen in OVA-sensitized mice
HERO ID:	1101921

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Room conditions reported, food and water ad libitum. Housing details not provided (but consistent with guidelines of Japanese Association for Laboratory Animal Science)
	Metric 15:	Number per Group	Medium	× 1	2	3-5/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Various immunological endpoints assessed in OVA-sensitized and non-sensitized mice from each exposure group; body weight and drinking water intake monitored.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Evaluated across all groups, including OVA sensitized and non-sensitized animals.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	3-5/group.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	All quantitative endpoints.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control data reported (both sensitized and non-sensitized). Non-sensitized animals showed consistent findings (regardless of exposure), as expected.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Initial BW not reported, but no exposure-related changes in BW. No exposure-related changes in drinking water intake.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	As indicated by study design, allergic reactions in OVA-sensitized controls were observed as expected. Study designed to determine if exposure enhanced allergic reactions, compared with control. No other observations unrelated to exposure reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	one-way ANOVA followed by Bonferroni's post-hoc procedure.
	Metric 24:	Reporting of Data	High	× 2	2	Immunological findings reported graphically. Qualitative reporting of BW and water intake (no exposure-related findings).
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	Kobayashi, R., Ikemoto, T., Seo, M., Satoh, M., Inagaki, N., Nagai, H., Nagase, H. (2010). Enhancement of immediate allergic reactions by trichloroethylene ingestion via drinking water in mice Journal of Toxicological Sciences, 35(5), 699-707
Data Type:	Immunotoxicity Screen in OVA-sensitized mice
HERO ID:	1101921

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 15: **Animal toxicity evaluation results of Boverhof et al 2013 for a 4-wk inhalation study in rats on mortality, nutrition and metabolic/adult exposure body weight, hematological and immune, hepatic, renal, and respiratory outcomes**

Study Citation:	Boverhof, D.R., Krieger, S.M., Hotchkiss, J., Stebbins, K.E., Thomas, J., Woolhiser, M.R. (2013). Assessment of the immunotoxic potential of trichloroethylene and perchloroethylene in rats following inhalation exposure Journal of Immunotoxicology, 10(3), 311-320
Data Type:	4-week inhalation (TCE)
HERO ID:	2127872

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported incompletely (a batch/lot number was not reported).
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was acceptable (reported to be 99.99% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative control group (filtered air only) was used and was appropriate.
	Metric 5:	Positive Controls	High	× 1	1	A positive control group (injected with cyclophosphamide) was included and was appropriate.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study authors did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance preparation and method and equipment used to generate the test substance as a vapor were reported and appropriate. The study authors did not report how the test substance was stored, so I downgraded the score to medium.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of the exposure administration were reported and exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity. Test concentrations in the chambers were analytically determined at least once per hour during the exposures and mean analytical concentrations were reported. The analytical method used to measure chamber concentrations was reported and appropriate.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for the study and outcomes of interest.

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Study Citation:	Boverhof, D.R., Krieger, S.M., Hotchkiss, J., Stebbins, K.E., Thomas, J., Woolhiser, M.R. (2013). Assessment of the immunotoxic potential of trichloroethylene and perchloroethylene in rats following inhalation exposure Journal of Immunotoxicology, 10(3), 311-320
Data Type:	4-week inhalation (TCE)
HERO ID:	2127872

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and concentration spacing were justified by the study authors (based on previous studies/animal data) and considered adequate to address the purpose of the study.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance. A dynamic whole body chamber was used and acceptable for the test substance vapor.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and age were reported and the test animals were obtained from a commercial source. Initial body weights and health status at the start of the study were not reported although the animals were certified Virus Antibody Free by the source. Due to reporting deficiencies, I downgraded the score to medium.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and if differences occurred between control and exposed groups.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per group (8 females/dose group) was less than the typical number used in studies of the same or similar type (e.g., subchronic toxicity study).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcomes of interest and was sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported and the study used adequate sampling for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were reported.
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the negative control group was reported and acceptable.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not reported to have been evaluated in this inhalation study; however, TCE is a potential respiratory irritant so I downgraded the score to low.

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Study Citation:	Boverhof, D.R., Krieger, S.M., Hotchkiss, J., Stebbins, K.E., Thomas, J., Woolhiser, M.R. (2013). Assessment of the immunotoxic potential of trichloroethylene and perchloroethylene in rats following inhalation exposure Journal of Immunotoxicology, 10(3), 311-320
Data Type:	4-week inhalation (TCE)
HERO ID:	2127872

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for the datasets.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data for most exposure-related findings were reported for most, but not all, outcomes by exposure group. However, some exposure-related data were not reported quantitatively (e.g., reduced body weights).
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 16: **Animal toxicity evaluation results of Seo et al 2012 for a 2- to 4-wk drinking water exposure study in mice on hematological and immune outcomes**

Study Citation:	Seo, M., Kobayashi, R., Okamura, T., Ikeda, K., Satoh, M., Inagaki, N., Nagai, H., Nagase, H (2012). Enhancing effects of trichloroethylene and tetrachloroethylene on type I allergic responses in mice Journal of Toxicological Sciences, 37(2), 439-445					
Data Type:						
HERO ID:	2128339					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	
	Metric 2:	Test Substance Source	High	× 1	1	
	Metric 3:	Test Substance Purity	High	× 1	1	
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent control did not receive vehicle (DMSO) but author states that this concentration of DMSO did not have effects in preliminary experiments.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control is not required.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups. Some experiments were done on cells isolated from animals.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The storage of the chemical was not stated, but it is not known to be unstable (WI).
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	The drinking water dosing was changed every other day, not every day. The concentration was below the solubility, but the test compound is slightly volatile.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Nominal drinking water concentrations are provided and doses are presented as mean ug ingested per day by each group of 8 mice (not adjusted for body weight). Also, it is unclear if water intake varied among treatment groups. The IP dose injections and the in vitro doses were defined.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The dosing was in drinking water ad libitum, but the duration was defined.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Dose spacing was 10-100 fold.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Test substance if volatile, but drinking water was changed every other day.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Mouse strains were identified. Body weight and health status were not reported.

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Study Citation: Seo, M., Kobayashi, R., Okamura, T., Ikeda, K., Satoh, M., Inagaki, N., Nagai, H., Nagase, H (2012). Enhancing effects of trichloroethylene and tetrachloroethylene on type I allergic responses in mice Journal of Toxicological Sciences, 37(2), 439-445

Data Type:

HERO ID: 2128339

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Minimal details on husbandry conditions were provided. The dietary mix was not identified.
	Metric 15:	Number per Group	Unacceptable	× 1	4	The number of animals per study group was not reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcomes were consistent across experiments.
	Metric 18:	Sampling Adequacy	Low	× 1	3	It is not clear what the experimental unit was (i.e., whether the outcome was measured separately for each individual animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Outcome was not subjective. The measurements used analytical devices.
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Water intake was not reported separately for each dose group, so it is unclear whether there were differences in water intake among doses. The in vitro study and the IP study designs were better controlled.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Heath outcomes unrelated to exposure were not reported; however, no differences in health among study groups were reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Limited details regarding statistics were provided. Graphs were plotted for the results, but the numerical raw data was not provided.
	Metric 24:	Reporting of Data	High	× 2	2	
Overall Quality Determination‡			Unacceptable⋆⋆		1.8	
Extracted			Yes			

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Study Citation:	Seo, M., Kobayashi, R., Okamura, T., Ikeda, K., Satoh, M., Inagaki, N., Nagai, H., Nagase, H (2012). Enhancing effects of trichloroethylene and tetrachloroethylene on type I allergic responses in mice Journal of Toxicological Sciences, 37(2), 439-445
Data Type:	
HERO ID:	2128339

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 17: **Animal toxicity evaluation results of Kobayashi et al 2012 for an in vivo 2-wk immunotoxicity study in mice on hematological and immune outcomes**

Study Citation:	Kobayashi, R; Nakanishi, T; Nagase, H (2012). Trichloroethylene enhances TCR-CD3-induced proliferation of CD8(+) rather than CD4(+) T cells Journal of Toxicological Sciences, 37(2), 381-387					
Data Type:	in vivo 2-week immunotoxicity study in mice					
HERO ID:	2128788					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance clearly identified
	Metric 2:	Test Substance Source	Medium	× 1	2	Manufacturer was identified, but batch/lot number not specified.
	Metric 3:	Test Substance Purity	High	× 1	1	It was specified that all the reagents were analytical grade although the exact percent purity was not provided
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	An appropriate and concurrent negative control group was included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable based on study type
	Metric 6:	Randomized Allocation	Medium	× 1	2	The study did not report randomization, however, mice were divided into group of six and received treatment using test chemical that was freshly prepared daily.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation specified; storage not specified, but unlikely to impact the study
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	TCE doses were identified
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	the exposure frequency and duration of exposure was reported and appropriate for the study outcome.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Tested concentrations and spacing appear to be justified by previously performed studies; the number of exposure groups and concentration spacing are adequate to show results relevant to the outcome.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure was suited to the test substance.; drinking water solutions were prepared fresh daily to mitigate any volatility.
Domain 4: Test Organism						

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Study Citation:	Kobayashi, R; Nakanishi, T; Nagase, H (2012). Trichloroethylene enhances TCR-CD3-induced proliferation of CD8(+) rather than CD4(+) T cells Journal of Toxicological Sciences, 37(2), 381-387
Data Type:	in vivo 2-week immunotoxicity study in mice
HERO ID:	2128788

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species were obtained from a commercial source and strain, sex, and age were reported. Minor uncertainties in the reporting (starting body weight was not reported), however unlikely to have a substantial impact on results assuming they all came from the same batch. It was reported that they were 7-9 weeks old.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were adequately reported.
	Metric 15:	Number per Group	High	× 1	1	The reported number of animals/exposure group (6/group) is sufficient for the study type and is consistent with similar types of studies.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcome of interest (effect of TCE on splenocyte cell differentiation).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment protocol was reported and was conducted consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details for sampling for the outcome was reported and was adequate for the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not typically applicable to this study type; the outcome evaluated in this study was not subjective.
	Metric 20:	Negative Control Response	High	× 1	1	The response of the negative control groups were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	There was some lack of reporting for some confounding variables; although data for initial body weight and food/water consumption was not shown, there is no indication that there were differences in palatability (and body weight gains were similar across groups); the lack of reporting is not likely to have a significant impact on study results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on health outcomes unrelated to exposure for each study group was not reported, but is unlikely to have a substantial impact on results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analysis was described with some omissions, but would not be likely to have a substantial impact on results.

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Study Citation:	Kobayashi, R; Nakanishi, T; Nagase, H (2012). Trichloroethylene enhances TCR-CD3-induced proliferation of CD8(+) rather than CD4(+) T cells Journal of Toxicological Sciences, 37(2), 381-387
Data Type:	in vivo 2-week immunotoxicity study in mice
HERO ID:	2128788

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	High	× 2	2	Data was presented for all outcomes and exposure groups
Overall Quality Determination‡		High		1.3	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 18: **Animal toxicity evaluation results of Yoo et al 2015 for a 1 to 4 wk gavage study in two strains of mice on hepatic outcomes**

Study Citation:	Yoo, H; Bradford, BU; Kosyk, O; Shymonyak, S; Uehara, T; Collins, LB; Bodnar, WM; Ball, LM; Gold, A; Rusyn, I (2015). Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: Liver effects Journal of Toxicology and Environmental Health, Part A: Current Issues, 78(1), 15-31
Data Type:	1 to 4 week gavage study of liver effects in two strains of mice
HERO ID:	2799569

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance source was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Neither purity nor grade was reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham-treated negative controls received vehicle.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for this study type.
	Metric 6:	Randomized Allocation	High	× 1	1	Study reports random allocation.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Test substance preparation and storage were not reported.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Time of day of gavage administration was not reported, and TCE absorption is altered by fasting.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Doses were reported in mg/kg bw but initial body weights were not given.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate and sufficient to observe effects.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Two nonzero doses differing by 4-fold were used. The doses were sufficient to observe an effect on relative liver weight.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route was oral gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Initial body weight was not reported, but age was reported. Several strains were tested.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most animal husbandry conditions were reported, other than temperature and humidity. No deviations were reported.
	Metric 15:	Number per Group	Low	× 1	3	Group sizes were 3-4 per dose
Domain 5: Outcome Assessment						

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Study Citation:	Yoo, H; Bradford, BU; Kosyk, O; Shymonyak, S; Uehara, T; Collins, LB; Bodnar, WM; Ball, LM; Gold, A; Rusyn, I (2015). Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: Liver effects Journal of Toxicology and Environmental Health, Part A: Current Issues, 78(1), 15-31
Data Type:	1 to 4 week gavage study of liver effects in two strains of mice
HERO ID:	2799569

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The apical outcomes evaluated were relative liver weight and histopathology, Timing of the outcome evaluations was reported and appropriate. Other outcomes included metabolite levels, hepatocellular proliferation, and gene expression changes.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No deviations or inconsistencies in outcome assessment execution were reported.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding numbers of samples for outcome assessments were not reported. Numbers of animals treated were not reported, but numbers of animals included in the results were.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control response was reported and appeared to be acceptable.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial and final body weights and food intake were not reported, which could affect relative liver weight.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	No information on attrition was available. Numbers of animals treated were not reported, but numbers of animals included in outcome assessments were. Authors did not report information on health outcomes other than the measured ones.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was performed and described in detail, and appeared to be appropriate for the tests.
	Metric 24:	Reporting of Data	Low	× 2	6	Most data were reported graphically; body weight data were not reported and could be important in interpretation of relative liver weights. Histopathology results were reported qualitatively.
Overall Quality Determination[‡]			~~Unacceptable~~[⋆⋆] ⟶ Low[§]		~~2.1~~	
Extracted			Yes			

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Study Citation:	Yoo, H; Bradford, BU; Kosyk, O; Shymonyak, S; Uehara, T; Collins, LB; Bodnar, WM; Ball, LM; Gold, A; Rusyn, I (2015). Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: Liver effects Journal of Toxicology and Environmental Health, Part A: Current Issues, 78(1), 15-31
Data Type:	1 to 4 week gavage study of liver effects in two strains of mice
HERO ID:	2799569

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i \left(\text{Metric Score}_i \times \text{MWF}_i\right) / \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "The only metric for which study was unacceptable was 7 (failure to report preparation and storage conditions), which is less important for a gavage study than for diet/drinking water/inhalation."

Table 19: **Animal toxicity evaluation results of Yoo et al 2015 for a 5-day gavage study in mice on hepatic outcomes**

Study Citation:	Yoo, H; Bradford, BU; Kosyk, O; Shymonyak, S; Uehara, T; Collins, LB; Bodnar, WM; Ball, LM; Gold, A; Rusyn, I (2015). Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: Liver effects Journal of Toxicology and Environmental Health, Part A: Current Issues, 78(1), 15-31
Data Type:	5 day gavage study of liver effects in different strains of mice
HERO ID:	2799569

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance source was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Neither purity nor grade was reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham-treated negative controls received vehicle.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for this study type.
	Metric 6:	Randomized Allocation	High	× 1	1	Study reports random allocation.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Test substance preparation and storage were not reported.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Time of day of gavage administration was not reported, and TCE absorption is altered by fasting.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Doses were reported in mg/kg bw but initial body weights were not given.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate and sufficient to observe effects.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Unacceptable	× 1	4	Only one dose was used in the 5 day study; this does not meet PECO criteria
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route was oral gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Initial body weight was not reported, but age was reported. Several strains were tested.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most animal husbandry conditions were reported, other than temperature and humidity. No deviations were reported.
	Metric 15:	Number per Group	Low	× 1	3	Group sizes were 3-4 per dose
Domain 5: Outcome Assessment						

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Study Citation:	Yoo, H; Bradford, BU; Kosyk, O; Shymonyak, S; Uehara, T; Collins, LB; Bodnar, WM; Ball, LM; Gold, A; Rusyn, I (2015). Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: Liver effects Journal of Toxicology and Environmental Health, Part A: Current Issues, 78(1), 15-31
Data Type:	5 day gavage study of liver effects in different strains of mice
HERO ID:	2799569

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The only apical outcome evaluated was relative liver weight, Timing of the outcome evaluations was reported and appropriate. Other outcomes included metabolite levels, hepatocellular proliferation, and gene expression changes.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No deviations or inconsistencies in outcome assessment execution were reported.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding numbers of samples for outcome assessments were not reported. Numbers of animals treated were not reported, but numbers of animals included in the results were.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control response was reported and appeared to be acceptable.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial and final body weights and food intake were not reported, which could affect relative liver weight.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	No information on attrition was available. Numbers of animals treated were not reported, but numbers of animals included in outcome assessments were. Authors did not report information on health outcomes other than the measured ones.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was performed and described in detail, and appeared to be appropriate for the tests.
	Metric 24:	Reporting of Data	Low	× 2	6	Most data were reported graphically; body weight data were not reported and could be important in interpretation of relative liver weights.
Overall Quality Determination‡			Unacceptable⋆⋆		2.1	
Extracted			No			

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Study Citation:	Yoo, H; Bradford, BU; Kosyk, O; Shymonyak, S; Uehara, T; Collins, LB; Bodnar, WM; Ball, LM; Gold, A; Rusyn, I (2015). Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: Liver effects Journal of Toxicology and Environmental Health, Part A: Current Issues, 78(1), 15-31
Data Type:	5 day gavage study of liver effects in different strains of mice
HERO ID:	2799569

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 20: **Animal toxicity evaluation results of Yoo et al 2015 for a short-term oral gavage study in mice on renal outcomes**

Study Citation:	Yoo, H; Bradford, BU; Kosyk, O; Uehara, T; Shymonyak, S; Collins, LB; Bodnar, WM; Ball, LM; Gold, A; Rusyn, I (2015). Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: Kidney effects Journal of Toxicology and Environmental Health, Part A: Current Issues, 78(1), 32-49
Data Type:	Sub acute (5 days) and sub-chronic (upto 4 weeks) oral gavage study of renal effects in different strains of mice
HERO ID:	2799570

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance source was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Neither purity nor grade was reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	vehicle control was reported
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for this study type.
	Metric 6:	Randomized Allocation	Medium	× 1	2	Study reported random allocation for the sub-chronic (4 wk study) but not for the sub-acute (5-day study)
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Test substance preparation and storage were not reported.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Time of day of gavage administration was not reported, and TCE absorption is altered by fasting.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Doses were reported in mg/kg bw but initial body weights were not given.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were sufficient to observe effects.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	sub-acute study had single dose, but the sub-chronic study had two doses for 3 different weeks
	Metric 12:	Exposure Route and Method	High	× 1	1	exposure route was oral gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Initial body weight was not reported, but age was reported. Several strains of varying susceptibility were tested.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most animal husbandry conditions were reported, other than temperature and humidity. No deviations were reported.
	Metric 15:	Number per Group	Low	× 1	3	Group sizes were 3-4 per mouse strain.
Domain 5: Outcome Assessment						

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Study Citation:	Yoo, H; Bradford, BU; Kosyk, O; Uehara, T; Shymonyak, S; Collins, LB; Bodnar, WM; Ball, LM; Gold, A; Rusyn, I (2015). Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: Kidney effects Journal of Toxicology and Environmental Health, Part A: Current Issues, 78(1), 32-49
Data Type:	Sub acute (5 days) and sub-chronic (upto 4 weeks) oral gavage study of renal effects in different strains of mice
HERO ID:	2799570

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The only apical outcomes evaluated were relative kidney weight and BUN. Timing of the outcome evaluations was reported and appropriate. Other outcomes included metabolite levels, proximal tubule cell proliferation, and gene expression changes.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No deviations or inconsistencies in outcome assessment execution were reported.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding numbers of samples for outcome assessments were not reported. Numbers of animals treated were not reported, but numbers of animals included in the results were.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control response was reported and appeared to be acceptable.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial and final body weights were not reported, nor were food or water intake, which could affect renal endpoints such as BUN.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	No information on attrition was available. Numbers of animals treated were not reported, but numbers of animals included in outcome assessments were. Authors did not report information on health outcomes other than the measured ones.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was performed and described in detail, and appeared to be appropriate for the tests.
	Metric 24:	Reporting of Data	Medium	× 2	4	Most data were reported graphically; body weight data were not reported and could be important in interpretation of relative kidney weights.
Overall Quality Determination‡			Unacceptable⋆⋆		2.0	
Extracted			No			

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Study Citation:	Yoo, H; Bradford, BU; Kosyk, O; Uehara, T; Shymonyak, S; Collins, LB; Bodnar, WM; Ball, LM; Gold, A; Rusyn, I (2015). Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: Kidney effects Journal of Toxicology and Environmental Health, Part A: Current Issues, 78(1), 32-49
Data Type:	Sub acute (5 days) and sub-chronic (upto 4 weeks) oral gavage study of renal effects in different strains of mice
HERO ID:	2799570

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 21: **Animal toxicity evaluation results of U.S. EPA 2017 for a 1-day inhalation study in mice and rats on respiratory and neurological/behavioral outcomes**

Study Citation:	EPA (U.S. Environmental Protection Agency) (2017). Chemview. Substantial risk reports submitted by companies: Trichloroethylene
Data Type:	1 day inhalation - mouse and rat
HERO ID:	3996621

Domain	Metric		Rating†	MWF*	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	1,1,2-TCE (aristar grade)
	Metric 2:	Test Substance Source	Medium	× 1	2	BDH Chemicals Ltd. Batch/lot not reported. Independent analysis not conducted.
	Metric 3:	Test Substance Purity	High	× 1	1	Exact purity not reported, identified as "Aristar" grade. According to BDH Chemicals website, "The BDH ARISTAR® line of acids meet or exceed ACS specifications"
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Air-exposed controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not necessary for study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Atmospheres were generated into glass dessicators by passing vaporized TCE into the input airstream at 7 L/min.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Consistent exposure across study groups. Controls exposed to air only.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Only Target concentrations reported. Study authors note that atmospheric concentrations were continuously monitored by infrared analysis, but analytical concentrations were not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	6-hours; appropriate for acute inhalation study
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Mice - 4 exposure groups plus control Rats - 2 exposure groups plus control
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	No description of the inhalation chamber (only description of how vapor was generated).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Female CD-1 mice (20-25g, Charles River), female Alpk:ApfSD rats (ICI, body weight not reported). Age not reported.

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Study Citation: EPA (U.S. Environmental Protection Agency) (2017). Chemview. Substantial risk reports submitted by companies: Trichloroethylene
Data Type: 1 day inhalation - mouse and rat
HERO ID: 3996621

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Housed in temperature controlled room with 12-hr light/dark cycle. Humidity not reported. Food and water ad libitum (except during exposure). Housing/cages not described.
	Metric 15:	Number per Group	Medium	× 1	2	Number per group not clearly stated in methods. Based on data reporting, there were at least 6 mice/group and 3 rats/group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Respiratory: Lung weight, lung histology, enzyme activity in clara cells, lung microsomes, and cytosol Neuro: clinical signs
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Assessed in all groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Number per group not clearly stated in methods. Based on data reporting, there were at least 6 mice/group and 3 rats/group.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required for evaluated endpoints.
	Metric 20:	Negative Control Response	Medium	× 1	2	Quantitative control data not reported, but data for exposure groups reported as percent of control. Because only relative findings were reported, control values cannot be assessed for any deviation from the norm/expected.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial BW only reported for mice. No final BW data reported. However, this is not expected to impact results due to acute duration. Respiratory rate was not reported, but clinical signs were evaluated and mild anesthesia was reported in mice at higher concentrations. TCE causes little to no respiratory irritation at anesthetic concentrations.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	No health outcomes unrelated to exposure were reported (only clinical signs and respiratory endpoints were discussed). No deaths were reported. Attrition is not expected to be a factor in single-exposure study.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistics reported for enzyme activity, but statistical methods were not reported. Data reporting for rat enzyme activity are adequate for independent analysis, but not mouse. Neither quantitative data nor statistics were reported for other endpoints.

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Study Citation:	EPA (U.S. Environmental Protection Agency) (2017). Chemview. Substantial risk reports submitted by companies: Trichloroethylene
Data Type:	1 day inhalation - mouse and rat
HERO ID:	3996621

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	Low	× 2	6	Clinical signs reported qualitatively (including CS observed in high-exposure mice). Dose at which clinical signs were observed was not reported. Histopathological findings were reported qualitatively (including exposure-related effects observed in mice). Enzyme data were reported in graphs in terms of % control for mice (actual data not reported); quantitative data reported for rats.
Overall Quality Determination‡		Unacceptable⋆⋆		1.9	
Extracted		No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 22: **Animal toxicity evaluation results of Chemical et al 1981 for a 3-wk oral gavage (mice and rats) study on hepatic outcomes**

Study Citation:	Chemical Manufacturers Association (1981). Pharmacokinetics and micromolecular interactions of trichloroethylene in mice and rats as related to oncogenicity					
Data Type:	3-wk oral gavage (mice and rats)					
HERO ID:	4215564					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Test substance identified definitely.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported, including manufacturer, but the batch/lot number was not reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not required.
	Metric 6:	Randomized Allocation	High	× 1	1	Study authors report that "Animals were computer randomized into treatment groups in all repeated dosing experiments."
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The preparation of the test substance, but not its storage was reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Study reported the doses used.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The study reported the number of dose groups, but their spacing was not justified.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The source, species, strains, and sex were reported, however, age, initial body weight, and health status were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and if differences occurred between control and exposed populations.

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Study Citation:	Chemical Manufacturers Association (1981). Pharmacokinetics and micromolecular interactions of trichloroethylene in mice and rats as related to oncogenicity
Data Type:	3-wk oral gavage (mice and rats)
HERO ID:	4215564

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported, appropriate for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required.
	Metric 20:	Negative Control Response	High	× 1	1	Negative controls responded appropriately.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food/water intake, and respiratory rate were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes unrelated to exposure were observed.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Appropriate statistical analyses were used.
	Metric 24:	Reporting of Data	High	× 2	2	Data were presented for the outcomes of interest.
Overall Quality Determination[‡]			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 23: **Animal toxicity evaluation results of Kjellstrand et al 1983 for a short term neurotoxicity inhalation study on neurological/behavior outcomes**

Study Citation:	Kjellstrand, P; Holmquist, B; Alm, P; Kanje, M; Romare, S; Jonsson, I; Månsson, L; Bjerkemo, M (1983). Trichloroethylene: Further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice Acta Pharmacologica et Toxicologica, 53(5), 375-384					
Data Type:	short term neurotox - inhalation					
HERO ID:	65255					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Commercial trichloroethylene, stabilized with 0.01 % thymol and 0.03 % diisopropylamine
	Metric 2:	Test Substance Source	High	× 1	1	Manufactured by Billerud-Uddeholm AB, Skoghall, Sweden. Batch number not reported, independent identity analysis not reported. Batch number not needed since TCE does not vary in composition.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Not reported, but identified as commercial grade
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent air-exposed controls
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed for study type. Some neuro studies require positive controls, but not necessary for this design (automated analysis)
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Details regarding generation of the test substance were not given, but a prior publication was cited for exposure details (Kjellstrand et al. 1981) and may contain those details. Stock concentration (assumed 100%) was not reported, and the exposure system was not thoroughly described. The authors mentioned stabilizers mixed in with TCE.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Consistent exposure across groups. Exposure levels dropped in all exposure groups twice weekly when chambers were opened for changing of bedding, water and food.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Only target concentrations were reported. Study authors indicate that "In principle it is a dynamic system with an approximate +/-5 % range of random fluctuation at the highest concentration used and +/- 10% at the lowest"; however, analytical analysis of exposure levels were not reported.

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Study Citation:	Kjellstrand, P; Holmquist, B; Alm, P; Kanje, M; Romare, S; Jonsson, I; Månsson, L; Bjerkemo, M (1983). Trichloroethylene: Further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice Acta Pharmacologica et Toxicologica, 53(5), 375-384
Data Type:	short term neurotox - inhalation
HERO ID:	65255

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Exposure for only 1 hr/day for 13 d. But motor activity analyzed during each exposure, so it was looking for acute effects of exposure (and potential effects of cumulative exposure). Therefore 1 hr/d is considered appropriate. for this endpoint. The justification for only using 1hr exposure was not provided, however significant results were observed so this exposure duration was adequate for the endpoint. Another repeated dose-study for motor function would have been useful for comparison however.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 exposure groups, plus control. Because the two doses resulted in opposite outcomes, an additional dose would have been useful.
	Metric 12:	Exposure Route and Method	Low	× 1	3	A prior publication was cited for exposure details (Kjellstrand et al. 1981) . Only details in this report are that it was a dynamic, whole-body exposure chamber. Neither this or the 1981 publication provides any information on air changes or chamber size.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	White male NMRI mice (Anticimex, Sweden). Initial BWs not reported for these animal groups. Age not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	The animals were separated by sex and housed in groups of ten in transparent cages. Commercial laboratory mouse food (AB Astra Evos, Sweden) and water were freely available. The temperature was 22+/-2". A 12 hour lighting schedule was controlled automatically with half an hour of twilight at dawn and dusk. Humidity not reported.
	Metric 15:	Number per Group	High	× 1	1	20 males/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Automated measurement of motor activity (Doppler). Measurements made before, during, and after hour-long exposure. Downgraded to medium because only one neurobehavioral outcome was assessed.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation across study groups.

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Study Citation:	Kjellstrand, P; Holmquist, B; Alm, P; Kanje, M; Romare, S; Jonsson, I; Månsson, L; Bjerkemo, M (1983). Trichloroethylene: Further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice Acta Pharmacologica et Toxicologica, 53(5), 375-384
Data Type:	short term neurotox - inhalation
HERO ID:	65255

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	Medium	× 1	2	20 males/group. Information not provided on technical replicates for motor activity. Downgraded to medium
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not needed for endpoints assessed.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group(s) were adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Body weights not reported; likely not an issue for short duration. (body weight changes at similar concentrations for longer durations show BW effects <20%). Respiratory rate not evaluated, but TCE causes little or no irritation to the respiratory tract at anesthetic concentrations (HSDB).
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistics not reported. Graphical reporting of data not adequate for independent analysis. Effect is mostly qualitative.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data reported graphically (mean of 13 consecutive nights). Individual night data not reported.
Overall Quality Determination‡			Medium		1.9	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 24: **Animal toxicity evaluation results of Kjellstrand et al 1987 for a 24-dy inhalation neurotoxicity study on neurological/behavior outcomes**

Study Citation:	Kjellstrand, P; Kanje, M; Bjerkemo, M (1987). Regeneration of the sciatic nerve in mice and rats exposed to trichloroethylene Toxicology Letters, 38(1-2), 187-191
Data Type:	24-day inhalation neurotoxicity study
HERO ID:	75365

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Test substance was identified by name only.
	Metric 2:	Test Substance Source	Low	× 1	3	No information was provide about the source and batch/lot number of TCE.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported. The authors report that TCE was cleaning grade quality and contained stabilizers, the details of the exposure system and the solvent were presented in a previous publication (Kjellstrand et al. 1981).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Controls were exposed to air.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not used for neurotoxicity studies of this type.
	Metric 6:	Randomized Allocation	Low	× 1	3	Allocation was not discussed , No details were provided in Kjellstrand et al. 1981.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	No information was provided on preparation and storage of TCE used in the study.
	Metric 8:	Consistency of Exposure Administration	Unacceptable	× 1	4	Methods for generating chamber atmosphere were not given.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Measured concentrations were not reported.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Continuous exposure for 4 or 24 days.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Concentrations were based on a previous study.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	No description of the inhalation chamber.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Source of the test animal was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	Unacceptable	× 1	4	Authors say that "The number of animals in the different groups is given in Table I". But this information is missing from the publication.

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Study Citation:	Kjellstrand, P; Kanje, M; Bjerkemo, M (1987). Regeneration of the sciatic nerve in mice and rats exposed to trichloroethylene Toxicology Letters, 38(1-2), 187-191
Data Type:	24-day inhalation neurotoxicity study
HERO ID:	75365

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	It is unclear whether this method is sensitive fo neuronal regeneration.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of outcome assessment protocol were reported.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not reported, but outcomes were objective.
	Metric 20:	Negative Control Response	High	× 1	1	Control responses were consistent
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not measured
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The authors mention that "Student's t-test was used fro the statistical evaluation".
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were reported.
Overall Quality Determination‡			Unacceptable⋆⋆		2.3	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 25: **Animal toxicity evaluation results of Kan et al 2007 for a short-term inhalation study on reproductive outcomes**

Study Citation:	Kan, FW; Forkert, PG; Wade, MG (2007). Trichloroethylene exposure elicits damage in epididymal epithelium and spermatozoa in mice Histology and Histopathology, 22(9), 977-988					
Data Type:	Short-term inhalation					
HERO ID:	700340					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported, including manufacturer, but the batch/lot number were not reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity (> 99.5%) was reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors reported using an appropriate concurrent negative control group. Study authors state that "for each experiment control animals were housed in adjacent chamber and treated identically except that the TCE evaporating system was not connected to the air intake."
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	Medium	× 1	2	The study did not report how animals were allocated to study groups, or there were deficiencies regarding the allocation method that are likely to have a substantial impact on results.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	The authors did not report the preparation and storage of the test substance.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups in a scientifically sound manner.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The authors used a single dose of TCE (1000 ppm). They report daily concentration in the inhalation chamber ranging from 970 ppm to 1010 ppm.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration were reported.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups were reported but dose/concentration spacing is not applicable since they chose only one treated dose and one control group.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						

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Study Citation:	Kan, FW; Forkert, PG; Wade, MG (2007). Trichloroethylene exposure elicits damage in epididymal epithelium and spermatozoa in mice Histology and Histopathology, 22(9), 977-988
Data Type:	Short-term inhalation
HERO ID:	700340

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, health status, age, were reported, and the test animal was obtained from a commercial source. The starting body weight of the animals was not reported, but the starting age (80-90 days) at the start of the exposure was reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported. Authors only report the light-dark cycle. Authors reported temperature and humidity during treatment but did not report light-dark cycle.
	Metric 15:	Number per Group	Medium	× 1	2	The authors used 4 animals/group, which is less for a sub-chronic study.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcome(s) of interest and was sensitive for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcome(s) of interest were reported.
	Metric 19:	Blinding of Assessors	Low	× 1	3	The study did not report whether assessors were blinded to treatment group for subjective outcomes, and this deficiency is likely to have a substantial impact on results.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group(s) were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food/water intake, and respiratory rate were not reported. These deficiencies are likely to have a substantial impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group and this deficiency is likely to have a substantial impact on results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	The study generated only qualitative data (histopathology), hence statistical analysis is not applicable.

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Study Citation:	Kan, FW; Forkert, PG; Wade, MG (2007). Trichloroethylene exposure elicits damage in epididymal epithelium and spermatozoa in mice Histology and Histopathology, 22(9), 977-988
Data Type:	Short-term inhalation
HERO ID:	700340

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.
Overall Quality Determination‡		~~High~~ ⟶ Medium§		~~1.6~~	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "The study was used to develop a POD, however it only contains qualitative histopathology information without any statistical backing. It is more of a supporting mechanistic paper."

Table 26: **Animal toxicity evaluation results of Dow et. al 2000 for a 18-day drinking water study in rats on clinical chemistry/biochemical, hematological and immune outcomes**

Study Citation:	Dow, J; Green, T (2000). Trichloroethylene induced vitamin B(12) and folate deficiency leads to increased formic acid excretion in the rat Toxicology, 146(2-3), 123-136
Data Type:	18-day drinking water study in rats
HERO ID:	701799

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	trichloroacetic acid (primary metabolite of TCE).
	Metric 2:	Test Substance Source	Medium	× 1	2	commercial source identified, but did not provide lot/batch numbers
	Metric 3:	Test Substance Purity	Low	× 1	3	purity and/or grade of test substances were not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Results indicate that a control was used, but characterization of the control group were not reported.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for this study
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	There are deficiencies in reporting of test substance preparation and storage
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	administered continuously in drinking water with water consumption monitored
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	doses of 0.25, 0.5, 1, 5, and 1 g/L were reported, but only the 1 and 5 g/l doses were converted to daily equivalent doses of 76 and 322 mg/kg-day. No information on animal body weight or intake was provided.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	study duration was only 18 days
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	two exposure groups; dosing 0.5 and 1 g/l for 3 months
	Metric 12:	Exposure Route and Method	High	× 1	1	drinking water
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal characteristics were provided
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Humidity and temperature were not reported, but noted to be in temperature controlled rooms
	Metric 15:	Number per Group	Low	× 1	3	only 4 animals/group
Domain 5: Outcome Assessment						

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Study Citation:	Dow, J; Green, T (2000). Trichloroethylene induced vitamin B(12) and folate deficiency leads to increased formic acid excretion in the rat Toxicology, 146(2-3), 123-136
Data Type:	18-day drinking water study in rats
HERO ID:	701799

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	endpoint methodologies were reported
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	no major flows seen in consistency of outcome assessment
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	
	Metric 20:	Negative Control Response	High	× 1	1	No effect of controls on the study
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	lack of reporting - exact water intake was not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	statistical methods not clearly described
	Metric 24:	Reporting of Data	Medium	× 2	4	
Overall Quality Determination‡			~~Medium~~ ⟶ Low§		~~1.9~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "CK: Experimental design not adequately described (Rats were administered 250 - 5000 mg/L in the drinking water for 15 days. After 15 days, only one group was given a control diet supplemented with folate). Only 4 animals were used per group. The exact purity of the test compound was not provided. The end points were excretion of formic acid which was analyzed to determine exposure-related effects on folic acid metabolism (more of a mechanistic study). Also, only the 1 and 5 g/l doses were converted (by the authors) to daily equivalent doses of 76 and 322 mg/kg-day. No information on animal body weight or intake was provided."

3 Other

Table 27: **In vitro evaluation results for Nakai et al 1999 for dermal absorption of TCE**

Study Citation:	J. S. Nakai, P. B. Stathopulos, G. L. Campbell, I. Chu, A. Li-Muller, R. Aucoin (1999). Penetration of chloroform, trichloroethylene, and tetrachloroethylene through human skin Journal of Toxicology and Environmental Health, Part A: Current Issues, 58(3,3), 157-170
Data Type:	In vitro dermal absorption of TCE
HERO ID:	630816

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was definitively identified using established nomenclature.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source (Sigma Chemical) of radiolabeled test chemical was provided with details on specific activity.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity was not given; however, the specific activity of the 14C-radiololabeled compound was provided.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Negative controls were not necessary for this study type.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not necessary for this study type.
	Metric 6:	Assay Procedures	High	× 1	1	Methods were well described and appropriate, especially controlling for volatility.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	The preparation and storage of the radiolabeled test substance were not described.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	The concentration of the donor solution was measured each hour and replenished as required
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Specific activity was reported; additional study details were given in a previous publication.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Steady state permeability was determined following 8h exposure.
	Metric 12:	Exposure Route and Method	Not Rated	NA	NA	Determination of steady state permeability did not require multiple exposure groups; goal was to provide infinite dose exposure by replenishing the donor solution hourly.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	
Domain 4: Test Model						

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Study Citation:	J. S. Nakai, P. B. Stathopulos, G. L. Campbell, I. Chu, A. Li-Muller, R. Aucoin (1999). Penetration of chloroform, trichloroethylene, and tetrachloroethylene through human skin Journal of Toxicology and Environmental Health, Part A: Current Issues, 58(3,3), 157-170
Data Type:	In vitro dermal absorption of TCE
HERO ID:	630816

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	Test model was routinely used and source described; in vitro human skin preparation system, modified for evaluations of volatile compounds.
	Metric 15:	Number per Group	High	× 1	1	Mean Kp values estimated for 6 fresh tissue obtained from human abdomen and breast and for 4 frozen tissues for comparison. 3-5 cells/tissue.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Analysis of cumulative radiolabel in receptor fluid by scintillation counting
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistently assessed across tissues.
	Metric 18:	Sampling Adequacy	High	× 2	2	3-5 cells per tissue; 4-6 tissues used.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were assessed.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Both breast and abdominal skin samples were obtained from different donors.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	Analysis of radiolabel reduces the possibility of confounding unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Methods for calculating cumulative permeation, chemical flux and permeability coefficient were clearly described.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Scoring and evaluation criteria are not applicable to this method.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity is not relevant to the test method.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for individual tissue samples as well as mean +- SD for Kp.
Overall Quality Determination‡			High		1.2	
Extracted			No			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

4 Subchronic (30-90 days)

Table 28: **Animal toxicity evaluation results of Buben et al 1985 for a 6-wk gavage of TCE in mice study on hepatic outcomes**

Study Citation:	Buben, JA; O'Flaherty, EJ (1985). Delineation of the role of metabolism in the hepatotoxicity of trichloroethylene and perchloroethylene: A dose-effect study Toxicology and Applied Pharmacology, 78(1), 105-122
Data Type:	6 week gavage study of TCE in mice
HERO ID:	65239

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name. No CASRN reported, but unambiguous name is sufficient
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained commercially
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity not reported, but test substance reportedly distilled prior to use.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham-treated controls received corn oil vehicle.
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	High	× 1	1	Study reports random allocation to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation method was reported and appropriate (prepared fresh 2-3x/wk); stability of test material in vehicle was either not evaluated or not reported,. but not expected to be of concern given the frequency of preparation. The storage conditions of the stock solution were not reported
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details of administration (e.g., time of day) were not reported; no dosing errors were noted.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose volumes were adjusted based on individual animal body weights obtained 3x/week.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Animals were dosed 5 days/week for 6 weeks. The duration was sufficient to induce the effects of interest (hepatotoxicity).
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Study used 7 exposure groups plus control; overall range of doses was 100-fold; high dose was adequate to identify effect, but there are no dose groups in which no effects were seen. The lowest TCE dose of 100 mg/kg is probably a NOAEL, but histopathy was only evaluated at 400mg/kg and 1600 mg/kg (effects seen at both) so it is difficult to determine the NOAEL

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Study Citation:	Buben, JA; O'Flaherty, EJ (1985). Delineation of the role of metabolism in the hepatotoxicity of trichloroethylene and perchloroethylene: A dose-effect study Toxicology and Applied Pharmacology, 78(1), 105-122
Data Type:	6 week gavage study of TCE in mice
HERO ID:	65239

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route and method were appropriate for the study type and test material.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal source, strain, sex, and age were reported. The ages of mice at study initiation varied between 3 and 5 months; however, as mice are adult at these ages, the age range is not expected to influence hepatotoxicity. A two-month spread in ages is not a concern, especially since animals were randomly allocated.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Temperature and light-dark cycle, and housing conditions were reported and appropriate, but humidity was not reported.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per group was reported, and generally consistent (most groups were 12-15 animals) with studies of this type, but the number per group varied with dose, and the highest dose groups had small numbers (4-6) per group. The lowest dose group for TCE also had only 4-6 mice.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Study focused on hepatotoxicity based on organ weight, liver G6P activity and triglycerides, serum ALT, and histopathology.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Study did not report any inconsistencies in execution of outcome assessments.. Histopathy was only reported in two dose groups
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Incomplete information was provided on sampling adequacy across endpoints. HIstopathology examinations were performed on controls, high dose animals, and on animals of one intermediate dose group.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	
	Metric 20:	Negative Control Response	High	× 1	1	Responses of negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Study did not report any potential differences among study groups that might influence the assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences among groups unrelated to exposure
Domain 7: Data Presentation and Analysis						

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Study Citation:	Buben, JA; O'Flaherty, EJ (1985). Delineation of the role of metabolism in the hepatotoxicity of trichloroethylene and perchloroethylene: A dose-effect study Toxicology and Applied Pharmacology, 78(1), 105-122
Data Type:	6 week gavage study of TCE in mice
HERO ID:	65239

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and appeared to be appropriate.
	Metric 24:	Reporting of Data	Medium	× 2	4	Histopathology results were reported semiquantitatively (incidences not reported); no statistical analysis of incidences was performed, and the available data are not adequate to perform independent statistical analysis. Data was quantitatively reported for all outcomes other than histopathy at all dose groups. Upgraded to medium.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 29: **Animal toxicity evaluation results of Gilbert et al 2011 for a 8-wk drinking water exposure study on liver, kidney, skin and connective tissue, body weight, hematological, and immune outcomes**

Study Citation:	Gilbert, K.M., Rowley, B., Gomez-Acevedo, H., Blossom, S.J. (2011). Coexposure to mercury increases immunotoxicity of trichloroethylene Toxicological Sciences, 119(2), 281-292
Data Type:	8-week drinking water exposure
HERO ID:	2127985

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance is identified by chemical name.
	Metric 2:	Test Substance Source	High	× 1	1	Source of test substance is reported (Aldrich Chemical Co).
	Metric 3:	Test Substance Purity	High	× 1	1	Purity of 99+%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Use of a concurrent negative control group is reported ("water alone"). 1% emulsifier was used to prepare drinking water containing TCE, but it is not clear if control drinking water also contained 1% emulsifier. This is not likely to have a substantial impact on interpretation of results.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control group was not necessary.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups. It is unknown whether this had a substantial impact on results.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Drinking water was prepared every 2-3 days, rather than every day. Test substance is volatile, so daily preparation of drinking water would be preferred.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	The test substance was prepared in the same way for both study groups. Drinking water was provided ad lib.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Authors provide calculated doses of 9.9 and 186.9 mg/kg/day, based on "water intake, body weight, and measured TCE degradation in the water bottles." The authors do not describe how TCE degradation was measured. It is unclear if drinking water concentrations were measured analytically.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Drinking water was provided ad lib.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The two dose groups were spaced far apart. Exposure concentrations were high enough to see effects on some outcomes of interest. Authors note that the doses straddle the 8-hour PEL by OSHA of approximately 76 mg/kg/day.

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Study Citation:	Gilbert, K.M., Rowley, B., Gomez-Acevedo, H., Blossom, S.J. (2011). Coexposure to mercury increases immunotoxicity of trichloroethylene Toxicological Sciences, 119(2), 281-292
Data Type:	8-week drinking water exposure
HERO ID:	2127985

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The exposure route (drinking water) was not the best option due to volatility of TCE.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Unacceptable	× 2	8	The strain (MRL +/+) is autoimmune-prone and should not be used for effects that do not pertain to autoimmunity (non-immune effects are the only effects being evaluated on this form, since the mechanistic immunotoxicity results of this study do not meet the PECO criteria). In addition, initial body weights of mice were not reported. Age was 8 weeks at study initiation. Only females were used. Mice were from Jackson Laboratories.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported. Information on temperature, humidity, and light-dark cycle of housing was not provided.
	Metric 15:	Number per Group	Medium	× 1	2	Number of animals was 6 per group. Not all animals were used for all outcomes (3/group used for gene expression). Some standard deviations are large.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment appeared to be consistent across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	The number of animals/group was suboptimal as already noted in Metric 15. However, with the exception of gene expression, the study appeared to include all animals/group for each outcome assessed.
	Metric 19:	Blinding of Assessors	High	× 1	1	Although blinding is not required for initial histopathological review, the authors report that histological changes were scored in a blinded manner. All other outcomes assessed are not subjective.
	Metric 20:	Negative Control Response	High	× 1	1	Control response appears acceptable.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	There was reduced water consumption at the higher TCE concentration, but this appeared to be factored into the dose calculations.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	There did not appear to be differences in health outcomes among groups that were unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis appeared appropriate.

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Study Citation:	Gilbert, K.M., Rowley, B., Gomez-Acevedo, H., Blossom, S.J. (2011). Coexposure to mercury increases immunotoxicity of trichloroethylene Toxicological Sciences, 119(2), 281-292
Data Type:	8-week drinking water exposure
HERO ID:	2127985

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	High	× 2	2	Data were presented for all outcomes.
Overall Quality Determination‡		Unacceptable⋆⋆		1.7	
Extracted		No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 30: **Animal toxicity evaluation results of Liu et al 2010 for a 6-wk oral gavage study in rats on neurological/behavior outcomes**

Study Citation:	Liu, M., Choi, D.Y., Hunter, R.L., Pandya, J.D., Cass, W.A., Sullivan, P.G., Kim, H.C., Gash, D., Bing, G. (2010). Trichloroethylene induces dopaminergic neurodegeneration in Fisher 344 rats Journal of Neurochemistry, 112(3), 773-783
Data Type:	6 week oral gavage study of neurodegeneration in rats
HERO ID:	2128146

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance source was reported, but without certification or analytical verification of identity.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity reported as = 99.5%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham-treated concurrent negative control group received vehicle.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not typical for study type
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Test substance preparation and storage conditions were not reported.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Study did not report time of day of gavage administration; TCE absorption is affected by fasting.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Study reported gavage doses in mg/kg bw but did not indicate body weights used to calculate dose or the frequency with which body weights were measured, or whether dosing materials were adjusted. with changes in body weight. However, the animals were 5 months of age and thus not in a growth phase, so body weight changes may not have been significant.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Frequency and duration were reported and suited to the study type/outcome. Duration was long enough to induce an effect.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	1 to 3 nonzero dose groups were used, depending on endpoint/experiment; spacing and range were sufficient to identify effect levels and no effect levels.
	Metric 12:	Exposure Route and Method	High	× 1	1	Route and method were reported and appropriate to the study type.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Test animal source not reported

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Study Citation:	Liu, M., Choi, D.Y., Hunter, R.L., Pandya, J.D., Cass, W.A., Sullivan, P.G., Kim, H.C., Gash, D., Bing, G. (2010). Trichloroethylene induces dopaminergic neurodegeneration in Fisher 344 rats Journal of Neurochemistry, 112(3), 773-783
Data Type:	6 week oral gavage study of neurodegeneration in rats
HERO ID:	2128146

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 14: Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were not reported.
	Metric 15: Number per Group	Medium	× 1	2	Group sizes ranged between 6 and 9 depending on the endpoint evaluated.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	Outcome assessment was described in detail and evaluated sensitive endpoints (immunohistochemistry in brain, rotarod testing, mitochondrial activity)
	Metric 17: Consistency of Outcome Assessment	Medium	× 1	2	Some important details of outcome assessment protocol execution were not reported (e.g., time of day of rotarod testing and timing of training vs test runs); however, these limitations do not affect all endpoints.
	Metric 18: Sampling Adequacy	Medium	× 1	2	With the exception of mitochondrial activity in the substantia nigra (for which pooled samples were used), sample sizes ranging from 6-9 animals were evaluated for each endpoint.
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	Blinding not typical for initial histopathology or objective endpoints.
	Metric 20: Negative Control Response	High	× 1	1	There were no apparent issues with the control responses.
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weights, body weight changes, and food and water intake were not reported but are not expected to significantly impact the results
	Metric 22: Health Outcomes Unrelated to Exposure	Low	× 1	3	No health outcomes apart from the primary measurements were reported.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Statistical analysis was performed; methods reported were appropriate for the data.
	Metric 24: Reporting of Data	Medium	× 2	4	Data were reported graphically for most endpoints; incidences of histologic changes were not reported.
Overall Quality Determination‡		~~Unacceptable~~⋆⋆ ⟶ Medium§		~~1.8~~	
Extracted		Yes			

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Study Citation:	Liu, M., Choi, D.Y., Hunter, R.L., Pandya, J.D., Cass, W.A., Sullivan, P.G., Kim, H.C., Gash, D., Bing, G. (2010). Trichloroethylene induces dopaminergic neurodegeneration in Fisher 344 rats Journal of Neurochemistry, 112(3), 773-783
Data Type:	6 week oral gavage study of neurodegeneration in rats
HERO ID:	2128146

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "The only metric for which the study was unacceptable was Metric 7 (preparation and storage); otherwise the study was well-conducted. It was upgraded because it measures sensitive endpoints (effects on substantia nigra) in the rat corroborating effects reported in humans exposed occupationally."

Table 31: **Animal toxicity evaluation results of Gilbert et al 2014 for a 12-wk immunotoxicity (mouse) study on body weight, hematological, and immune outcomes**

Study Citation:	Gilbert, KM; Reisfeld, B; Zurlinden, TJ; Kreps, MN; Erickson, SW; Blossom, SJ (2014). Modeling toxicodynamic effects of trichloroethylene on liver in mouse model of autoimmune hepatitis Toxicology and Applied Pharmacology, 279(3), 284-293
Data Type:	12-wk immunotoxicity study
HERO ID:	2799650

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE
	Metric 2:	Test Substance Source	Medium	× 1	2	Aldrich Chemical; batch no. not reported, no independent analytical analysis
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was provided (99+%)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent control used. Unclear if it was an untreated control or a vehicle (1% emulsifier Alkamuls EL-620).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed for study type
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Suspended in drinking water with 1% emulsifier Alkamuls EL-620. Freshly made every 2-3 days. Storage not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Drinking water solutions replaced every 2-3 days. Unclear if controls were exposed to vehicle (did not downgrade here - downgraded from high to medium in Metric 4)
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Only target water concentrations reported; no analytical analysis of drinking water solutions. Body weight and water consumption were measured, but only qualitatively reported (no exposure-related changes), so actual doses cannot be calculated. Doses in mg/kg-d can be estimated using reference body weight and drinking water intake; however, since this is a non-standard strain there would be additional uncertainty.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	12-wks for the multi dose study and multi-weeks for the single dose study, water ad libitum
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	3 doses plus control
	Metric 12:	Exposure Route and Method	High	× 1	1	Drinking water with emulsifier, replaced every 2-3 d

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Study Citation: Gilbert, KM; Reisfeld, B; Zurlinden, TJ; Kreps, MN; Erickson, SW; Blossom, SJ (2014). Modeling toxicodynamic effects of trichloroethylene on liver in mouse model of autoimmune hepatitis Toxicology and Applied Pharmacology, 279(3), 284-293
Data Type: 12-wk immunotoxicity study
HERO ID: 2799650

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Unacceptable	× 2	8	The strain (MRL +/+) is autoimmune-prone and should not be used for effects that do not pertain to autoimmunity (non-immune effects are the only effects being evaluated on this form, since the mechanistic immunotoxicity results of this study do not meet the PECO criteria). NK: Not an expert with respect to this strain of mice but assume it is autoimmune-prone and not a suitable strain for the part of study that is being evaluated.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry details limited to housed in polycarbonate ventilated cages and provided with drinking water ad libitum. No details provided regarding room conditions or diet (although all studies were approved by the Animal Care and Use Committee at the University of Arkansas for Medical Sciences).
	Metric 15:	Number per Group	High	× 1	1	12 females/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Body weight: weekly Immunotoxicity: cytokine analysis (macrophage activity, gene expression), antibody production Hepatic: Histopathology
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	According to methods, all endpoints assessed in all groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	High	× 1	1	Assessors were blind for histological assessment; other endpoints are quantitative.
	Metric 20:	Negative Control Response	Medium	× 1	2	It is assumed that control findings were standard for this strain (susceptible to autoimmune disease), but since a nonsusceptible strain was not used, this is unclear.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial BW not reported, but no exposure-related changes in body weight or water consumption were observed (reported qualitatively).

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Study Citation:	Gilbert, KM; Reisfeld, B; Zurlinden, TJ; Kreps, MN; Erickson, SW; Blossom, SJ (2014). Modeling toxicodynamic effects of trichloroethylene on liver in mouse model of autoimmune hepatitis Toxicology and Applied Pharmacology, 279(3), 284-293
Data Type:	12-wk immunotoxicity study
HERO ID:	2799650

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Since mouse strain is susceptible to autoimmune hepatitis, immunological findings in controls represent deviations from the norm. A non-susceptible strain was not used for comparison.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Analysis of variance (ANOVA) with subsequent t-test. Homogeneity of variance between groups was tested using the studentized Breusch–Pagan test, and normality of residuals using the Shapiro–Wilk test.Where significant deviations fromhomoscedasticity or normality were observed, the non-parametric Kruskal–Wallis and Wilcoxon rank sum tests were applied instead of ANOVA/t-test.
	Metric 24:	Reporting of Data	Low	× 2	6	Liver histology was not reported for 12-week study. Body weight data reported qualitatively (no effect). Other effects are mechanistic immunotoxicological effects that are not included in the PECO.
Overall Quality Determination‡			Unacceptable⋆⋆		1.9	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 32: **Animal toxicity evaluation results of U.S. EPA 2017 for a 13-wk inhalation study in rats on neurological/behavior and body weight outcomes**

Study Citation:	EPA (U.S. Environmental Protection Agency) (2017). Chemview. Substantial risk reports submitted by companies: Trichloroethylene					
Data Type:	13-wk neurotox inhalation study - rats					
HERO ID:	3996621					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test Substance Identity as TCE
	Metric 2:	Test Substance Source	High	× 1	1	Dow, batch not reported, but identity of sample was confirmed by infrared spectroscopy
	Metric 3:	Test Substance Purity	High	× 1	1	99.22% purity. The purity of the test material was determined by gas-chromatography
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Controls were exposed to air alone
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are required for neurotoxicity testing by OPPTS, but not OECD (positive controls are discussed but not required). No specific guideline was cited for this study. For OPPTS, they don't always have to be concurrent in an established lab with reliable historical data. This study does not report positive controls, but indicates that the rat strain selected has historical data (but data were not discussed).
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Detailed description of vaporization methods. Storage not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Similar conditions were used for control chambers with the exception of the test material. Analytical concentration was monitored 1-2 times/hour.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and analytical concentrations reported and averages were within 1% of target. Vapor distribution analysis was conducted at 6 pts within the animal breathing zone
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	13 wks, 5d/wk, 6 hr/d
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	3 doses plus control. Exposure levels selected based on prior testing. High dose was selected to be a "significant acute physiologic challenge to CNS" without resulting in excessive systemic toxicity that would compromise interpretability of the neurotox data.

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Study Citation: EPA (U.S. Environmental Protection Agency) (2017). Chemview. Substantial risk reports submitted by companies: Trichloroethylene
Data Type: 13-wk neurotox inhalation study - rats
HERO ID: 3996621

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	Whole-body inhalation chamber (Rochester-type, 2 cubic meters), dynamic air flow of 450 L/min. This would provide 13-14 air changes/hour.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	F344 rats; 14 wk old males and nulliparous and nonpregnant females. Selected due to general acceptance in neurotox testing and availability of historical data. All were determined to be in good health by lab vet. Pre-exposure body weights were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Housing and room conditions adequately described. Food and water available ad libitum except during exposure.
	Metric 15:	Number per Group	High	× 1	1	Exposed 10 out of 12 rats /sex/exposure group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Neurotox: Clinical signs, FOB (evaluated pre-exposure and at 4, 8, and 13 wk.) electrophysiology, neuropathology (control and high-dose) body weight
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent across groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	12/sex/group for BW, clinical signs, FOB 10-12/sex/group for electrophysiology 5/sex in control and high-dose for histology (but exposure-related effects observed in organ of corti)
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinded for FOB. Other endpoints were not subjective.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control data reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No exposure-related changes in body weight. Respiration was within normal limits during daily observations in all groups.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Some spontaneously occurring histopathological findings were reported, and considered not related to exposure (an minimal in severity). Study authors also discuss how non-neurological exposure related effects of exposure (e.g. lacrimation) could potentially alter behavior on neurological tests due to changes in sensory environement (as opposed to direct neurotoxic effect).
Domain 7: Data Presentation and Analysis						

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Study Citation:	EPA (U.S. Environmental Protection Agency) (2017). Chemview. Substantial risk reports submitted by companies: Trichloroethylene
Data Type:	13-wk neurotox inhalation study - rats
HERO ID:	3996621

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 23: Statistical Methods	High	× 1	1	Detailed reporting of statistical methods and outcomes of statistical tests.
	Metric 24: Reporting of Data	High	× 2	2	All data reported quantitatively.
Overall Quality Determination‡		High		1.2	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 33: **Animal toxicity evaluation results of Dow 1993 for a 13-wk inhalation study in rats on neurological/behavior outcomes**

Study Citation:	Dow Chemical Company (1993). Initial submission: Neurotoxicological examination of rats exposed to trichloroethylene vapor in 13 weeks with cover letter dated 100193 (sanitized)
Data Type:	13-week inhalation neurotoxicity in rats-behavior, electrodiagnostic
HERO ID:	4215753

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name, structure, mol weight, and physical chemical properties. Identity confirmed by infrared spectroscopy.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported and the identity was verified.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity was reported and determined by chromatography. Effects are likely to be to be due to the test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative control group was used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The method and chamber used to generate the vapor was described, but some details were omitted including how air was heated and how TCE was metered.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and/or outcome(s) of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Concentrations were justified and the spacing was appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The source, species, strain, age, sex, health status, and pre-exposure body weight were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported.

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Study Citation:	Dow Chemical Company (1993). Initial submission: Neurotoxicological examination of rats exposed to trichloroethylene vapor in 13 weeks with cover letter dated 100193 (sanitized)
Data Type:	13-week inhalation neurotoxicity in rats-behavior, electrodiagnostic
HERO ID:	4215753

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 15: Number per Group	High	× 1	1	The number of animals per group (10/sex/dose) is reported and appropriate for the study type.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was well described and addressed the outcomes of interest.
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18: Sampling Adequacy	High	× 1	1	Adequate sampling for the outcomes of interest was reported.
	Metric 19: Blinding of Assessors	High	× 1	1	The investigators assessing functional observational battery (FOB) were blinded to treatment. Electrodiagnostic testing did not require blinding.
	Metric 20: Negative Control Response	High	× 1	1	The negative control responses were adequate.
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences in initial body weight was reported. Food/water intake and respiratory rate were not reported.
	Metric 22: Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes unrelated to exposure were observed.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Statistical methods were described and appropriate.
	Metric 24: Reporting of Data	High	× 2	2	Data were reported for all outcomes.
Overall Quality Determination‡		High		1.2	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 34: **Animal toxicity evaluation results of Rohm Haas Co 1982 for a 13-wk oral exposure study in rats and mice on renal, hepatic, body weight, and mortality outcomes**

Study Citation:	Rohm & Haas Co (1982). Initial submission: Carcinogenesis bioassay with trichloroethylene in rats and mice (draft report) with cover letter dated 080392
Data Type:	13-week oral, rats, mice
HERO ID:	4215768

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name, CASRN, structure, formula, and mol. weight
	Metric 2:	Test Substance Source	High	× 1	1	The source and lot numbers were reported and identity analysis was conducted. The infrared and nuclear magnetic resonance spectra were reported to be consistent with literature spectra, although the data were not included in the pages of the document.
	Metric 3:	Test Substance Purity	High	× 1	1	The composition was such that effects likely due to test substance. The percentage of impurities and type of stabilizer used were identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative control group was used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control was not required for this assay.
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly assigned to cases and treatment group.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage information were reported and stability was tested.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	The volume received by rats and mice was administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Animals received the doses 5 d/wk for 13 weeks which is less than typical treatment of 7 d/wk for gavage studies. CK: Duration is adequate
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Although not justified, the number and spacing of groups was adequate for the purpose of the test.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route and method were suited to the test substance based on reported results of the stability tests..
Domain 4: Test Organism						

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Study Citation:	Rohm & Haas Co (1982). Initial submission: Carcinogenesis bioassay with trichloroethylene in rats and mice (draft report) with cover letter dated 080392
Data Type:	13-week oral, rats, mice
HERO ID:	4215768

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The source, strain, sex, age, and starting body weight were reported. The health status was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported and were appropriate.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was reported and appropriate for the study.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcomes methodology assessment addressed outcomes.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric was not required for endpoints in this study.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses were appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were not reported differences.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes unrelated to exposure were observed.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical analysis was not conducted but data were provided to conduct independence analysis for some endpoints.
	Metric 24:	Reporting of Data	Low	× 2	6	Data for dose-related findings were not reported for all exposure groups.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 35: **Animal toxicity evaluation results of NTP 1988 for a 13-wk oral toxicity study on mortality, nutrition and metabolic/adult exposure body weight, hepatic, renal, respiratory, reproductive, hematological and immune, endocrine, neurological/behavior, cardiovascular, skin and connective tissue, thyroid, and gastrointestinal outcomes**

Study Citation:	NTP (1988). Toxicology and carcinogenesis studies of trichloroethylene (CAS No. 79-01-6) in four strains of rats (ACI, August, Marshall, Osborne-Mendel) (gavage studies)
Data Type:	13-week oral toxicity
HERO ID:	65268

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance and lot numbers were reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was reported and sufficiently high such that the study results were likely to be due to the test substance itself. Impurities totaled less than 0.04%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate control group.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control is not indicated by the study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The study authors reported that animals were randomly allocated into study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation and storage conditions were reported and appropriate for the test substance.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were reported and methods were consistent among the groups. However, the gavage volumes slightly exceeded those typically used (e.g., 1 mL/100 g body weight). The dose volume was 1 mL in all three rat strains (ACI, August, and Marshall), which had mean initial body weights below 100 g in all groups except male Marshall rats and this volume slightly exceeded dosing volumes in similar studies until weights reached 100 g. Initial starting body weights were above 100g for all rat species. The reviewer's conclusion is incorrect. However, 14% of stock doses differed by more than 10% from target concentration.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.

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Study Citation: NTP (1988). Toxicology and carcinogenesis studies of trichloroethylene (CAS No. 79-01-6) in four strains of rats (ACI, August, Marshall, Osborne-Mendel) (gavage studies)
Data Type: 13-week oral toxicity
HERO ID: 65268

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for the study type and outcomes of interest. The test substance was administered orally 5 days/week for 13 weeks.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of dose groups and dose spacing were considered adequate to address the purpose of the study. Although the study authors did not justify the selection of doses, previous studies were reviewed in the introduction, and may have been considered for the selection of doses.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure (oral, gavage) were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal source, species, strain, sex, and starting body weight were reported. It is unclear whether age or health status were evaluated at the beginning of the study. The test animals were an appropriate animal model for evaluation of the specified outcomes of interest.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and if differences occurred between controls and test substance exposed groups (temperature and humidity were not reported; light-dark cycle duration was reported). Some details in the room conditions were not recorded, but food and water were provided ad libitum and it is assumed that all groups were stored totgether.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group (10/sex/group) was reported and appropriate for the study type and outcome analysis and consistent with studies of the same or similar type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology addressed the outcomes of interest (endpoints were limited to mortality, clinical signs, body weight, histopathology) and was sensitive for the outcomes of interest. Protocol was not described for histopathological assessment.

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Study Citation: NTP (1988). Toxicology and carcinogenesis studies of trichloroethylene (CAS No. 79-01-6) in four strains of rats (ACI, August, Marshall, Osborne-Mendel) (gavage studies)
Data Type: 13-week oral toxicity
HERO ID: 65268

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Details regarding the execution of the study protocol for outcome assessment were not reported for some outcomes (e.g., histology preparation, tissues examined). Absence of protocol was partially accounted for in metric 16. It is assumed that all groups were measured sacrificed at the same time, so outcomes should be relatively consistent.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported and the study used adequate sampling for the outcomes of interest. Although histopathology examination was limited to controls and high-dose animals, no histopathological changes related to the test substance were observed.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were reported and histopathology was not described as a re-evaluation so this metric is considered not applicable.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were adequate (e.g., survival, mean body weight gain).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among the study groups in initial body weight. While food and water intakes were not reported, this is not expected to have a significant impact on the results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	No statistical analyses were reported. Data were provided that would allow an independent statistical analysis (e.g., for mortality and some body weight data). Only summary statistics were provided for body weight and survival, without any error provided. Data was not provided for histopathy. Downgraded to low.

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Study Citation:	NTP (1988). Toxicology and carcinogenesis studies of trichloroethylene (CAS No. 79-01-6) in four strains of rats (ACI, August, Marshall, Osborne-Mendel) (gavage studies)
Data Type:	13-week oral toxicity
HERO ID:	65268

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	Medium	× 2	4	Data for exposure-related findings were presented for all outcomes. Negative findings were presented qualitatively and/or quantitatively. Data was not reported for histopath findings, although there was not much to present since it was negative. Individual data was also not provided.
Overall Quality Determination‡		~~High~~ ⟶ Medium§		~~1.4~~	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "The 13-week study is only a preliminary range-finding study for the 2-year study. Therefore, very little data is reported. This study would be a low based on the outcome assessment and data reporting, but all metrics related to the substance and methods were High."

Table 36: **Animal toxicity evaluation results of Isaacson et al 1990 for a 4-6-wk oral exposure study on neurological/behavior outcomes**

Study Citation:	Isaacson, LG; Spohler, SA; Taylor, DH (1990). Trichloroethylene affects learning and decreases myelin in the rat hippocampus Neurotoxicology and Teratology, 12(4), 375-381					
Data Type:	4-6-week neurological/behavior oral					
HERO ID:	65290					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively (by name).
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported; however, a batch/lot number was not provided.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative/vehicle control group (distilled water) was included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable - Positive control is not indicated for the study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The study authors reported that the animals were randomly assigned to treatment groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance in vehicle was reported, however, its storage was not reported. Also, either prepared solutions were stored between water bottle changes, which occurred approximately every 48 hours. Solutions in bottles were measured and reported to be similar for any 48-hour period.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details on exposure administration were reported and exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The authors estimated the average TCE concentrations in the bottles during any 48-hr period by gas chromatography. Based on the daily intake they reported the total dose of TCE consumed by the two different test groups. From previous studies (Ref. 20) the authors reported that there is no statistical difference in water consumption between treatment groups and control groups.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration were reported and were appropriate for the outcomes of interest.

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Study Citation:	Isaacson, LG; Spohler, SA; Taylor, DH (1990). Trichloroethylene affects learning and decreases myelin in the rat hippocampus Neurotoxicology and Teratology, 12(4), 375-381
Data Type:	4-6-week neurological/behavior oral
HERO ID:	65290

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	There were were deficiencies regarding the number of exposure groups and/or dose concentration spacing. This study is not designed for dose-response analysis. While earlier studies examined the pre- and post-natal exposure to TCE, this study deals with exposure of the young adult rats. The purpose is to examine the spacial learning effects of TCE exposure on animals exposed once with the effects on those receiving a second exposure.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Most test animal characteristics were reported, including species, strain, sex, life-stage, and source; however, starting body weight was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Some husbandry conditions, including temperature and light:dark cycle, were reported; however, humidity was not reported.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per study group (6 males/treatment group, Experiment 1 and 2) was reported. This number is lower than typical repeated exposure regimen studies (e.g., 8/sex/group); however, two experiments were performed, each with 6 males/group, and this number was sufficient for statistical analysis so I downgraded the score to medium rather than low.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcomes of interest and was sensitive for the outcomes of interest. Neurobehavioral function and histopathology of brain sections were conducted.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., at the same times following initial exposure).
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported and the study used adequate sampling for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Low	× 1	3	The study did not report whether assessors were blinded to treatment group for subjective outcomes (e.g., behavioral tests) and this deficiency could have a substantial impact on results.

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Study Citation: Isaacson, LG; Spohler, SA; Taylor, DH (1990). Trichloroethylene affects learning and decreases myelin in the rat hippocampus Neurotoxicology and Teratology, 12(4), 375-381
Data Type: 4-6-week neurological/behavior oral
HERO ID: 65290

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food intake were not reported, although the authors indicated no differences in water consumption (Ref 20). However, these reporting deficiencies may have a substantial impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The statistical methods were clearly described and appropriate for the datasets.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data for exposure-related findings were described in the text and data were only reported for some outcomes. For example, while histopathology of brain sections was conducted and treatment-related differences were found, data presentation was limited to four representative slide images (Figures 3A & 3B, 4A & 4B) and mean percent coverage of myelinated fibers in controls and TCE groups for one experiment (Table 2).
Overall Quality Determination‡			~~High~~ ⟶ Medium§		~~1.6~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study
§ Evaluator's explanation for rating change: "Doses were intermittent in group 2 and only a single consistent dose was applied, reducing the utility of the study for dose-response analysis."

Table 37: **Animal toxicity evaluation results of NCI 1976 for a 6-week gavage study in mice to establish MTD on mortality, nutrition and metabolic/adult exposure body weight outcomes**

Study Citation:	NCI (1976). Carcinogenesis bioassay of trichloroethylene					
Data Type:	6 week gavage study in mice to establish MTD					
HERO ID:	75178					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test material identified by unambiguous name
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained from manufacturer and identified by lot number and date of receipt
	Metric 3:	Test Substance Purity	High	× 1	1	Purity verified by GC and infrared spectroscopy to be at least 99%; impurities identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A sham-exposed negative control group received vehicle only.
	Metric 5:	Positive Controls	Not Rated	NA	NA	positive control is not typical
	Metric 6:	Randomized Allocation	Medium	× 1	2	Study reports that the animals were allocated randomly (albeit not by random number table) such that initial average weight of each group was approximately equal.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Details of preparation and storage were reported and should have been adequate to preserve TCE stability (e.g., prepared weekly, and stored in sealed and refrigerated containers), but stability was not tested.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	No variations in exposure administration across groups were reported.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Gavage volumes were not reported. In addition, dosing volume was determined by group mean body weight, so individual doses may have varied within a group if body weights varied.
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	Exposure duration was only 6 weeks; this is inadequate for subchronic exposure..
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Five nonzero doses were administered, with a range over 10-fold.
	Metric 12:	Exposure Route and Method	High	× 1	1	Gavage administration was reported and appropriate to the study type.
Domain 4: Test Organism						

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Study Citation: NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type: 6 week gavage study in mice to establish MTD
HERO ID: 75178

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animals were appropriate and obtained from commercial source, with strain, age, sex, and BW, but age at initiation of the 6 week study was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were reported in detail and no deviations were reported. The animals were housed in the same room with animals used in other experiments, but this is not expected to affect the results because both control and exposed groups were housed in the same room.
	Metric 15:	Number per Group	Medium	× 1	2	Group sizes were 5/sex/dose; this is consistent with recommendations for 28 day repeat dose studies but smaller than recommended for subchronic studies.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Endpoints were limited to mortality, body weight, clinical signs, and food intake. Gross necrospy was performed on animals 2 weeks after the end of exposure.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	There were no reported inconsistencies in outcome assessment across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals were sampled for all endpoints.
	Metric 19:	Blinding of Assessors	Low	× 1	3	Study did not report whether investigators evaluating clinical signs were blinded to treatment group.
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	No variations in test execution were reported, but few details were provided for the 6 week study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	No mice receiving the highest dose survived to the end of the study (2 weeks after end of exposure); there were also deaths in the next 2 highest dose groups.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analysis was not performed however, data enabling independent analysis (for body weight and mortality) were reported.
	Metric 24:	Reporting of Data	Medium	× 2	4	Incidences of clinical signs and gross necropsy findings were incompletely reported.
Overall Quality Determination[‡]			~~Medium~~ ⟶ Low[§]		~~1.7~~	
Extracted			Yes			

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Study Citation:	NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type:	6 week gavage study in mice to establish MTD
HERO ID:	75178

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "This is a 6-week study to establish a MTD; only body weight, mortality, clinical signs, and gross necropsy were evaluated, and the animals were necropsied after a 2 week postexposure observation period."

Table 38: **Animal toxicity evaluation results of NCI 1976 for a 6-week gavage study in rats to establish MTD on mortality, nutrition and metabolic/adult exposure body weight outcomes**

Study Citation:	NCI (1976). Carcinogenesis bioassay of trichloroethylene					
Data Type:	6 week gavage study in rats to establish MTD					
HERO ID:	75178					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test material identified by unambiguous name
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained from manufacturer and identified by lot number and date of receipt
	Metric 3:	Test Substance Purity	High	× 1	1	Purity verified by GC and infrared spectroscopy to be at least 99%; impurities identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A sham-exposed negative control group received vehicle only.
	Metric 5:	Positive Controls	Not Rated	NA	NA	positive control is not typical
	Metric 6:	Randomized Allocation	High	× 1	1	Study reports that the animals were allocated randomly (albeit not by random number table) such that initial average weight of each group was approximately equal.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Details of preparation and storage were reported and should have been adequate to preserve TCE stability (e.g., prepared weekly, and stored in sealed and refrigerated containers), but stability was not tested.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	No variations in exposure administration across groups were reported.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Gavage volumes were not reported. In addition, dosing volume was determined by group mean body weight, so individual doses may have varied within a group if body weights varied.
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	Exposure duration was only 6 weeks; this is inadequate for subchronic exposure..
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Five nonzero doses were administered, with a range over 10-fold.
	Metric 12:	Exposure Route and Method	High	× 1	1	Gavage administration was reported and appropriate to the study type.
Domain 4: Test Organism						

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Study Citation: NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type: 6 week gavage study in rats to establish MTD
HERO ID: 75178

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animals were appropriate and obtained from commercial source, with strain, age, sex, and BW, but age at initiation of the 6 week study was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were reported in detail and no deviations were reported. The animals were housed in the same room with animals used in other experiments, but this is not expected to affect the results because both control and exposed groups were housed in the same room.
	Metric 15:	Number per Group	Medium	× 1	2	Group sizes were 5/sex/dose; this is consistent with recommendations for 28 day repeat dose studies but smaller than recommended for subchronic studies.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Endpoints were limited to mortality, body weight, clinical signs, and food intake. Gross necropsy was performed on animals 2 weeks after the end of exposure.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	There were no reported inconsistencies in outcome assessment across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals were sampled for all endpoints.
	Metric 19:	Blinding of Assessors	Low	× 1	3	Study did not report whether investigators evaluating clinical signs were blinded to treatment group.
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	No variations in test execution were reported, but few details were provided for the 6 week study.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	No rats receiving the highest dose survived to the end of the study (2 weeks after end of exposure)
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analysis was not performed however, data enabling independent analysis (for body weight and mortality) were reported.
	Metric 24:	Reporting of Data	Medium	× 2	4	Incidences of clinical signs and gross necropsy findings were incompletely reported.
Overall Quality Determination[‡]			~~High~~ ⟶ Unacceptable[§]		~~1.7~~	
Extracted			Yes			

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Study Citation:	NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type:	6 week gavage study in rats to establish MTD
HERO ID:	75178

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study
§ Evaluator's explanation for rating change: "This is a 6-week study to establish a MTD; only body weight, mortality, clinical signs, and gross necropsy were evaluated, and the animals were necropsied after a 2 week postexposure observation period."

Table 39: **Animal toxicity evaluation results of NTP 1990 for a 13-wk oral study in rats and mice on mortality, nutrition and metabolic/adult exposure body weight outcomes**

Study Citation:	NTP (1990). Carcinogenesis studies of trichloroethylene (without epichlorohydrin) (CAS No. 79-01-6) in F344/N rats and B6C3F1 mice (gavage studies) Technical Report Series, 243					
Data Type:	13-week oral (rats and mice)					
HERO ID:	87574					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	High	× 1	1	
	Metric 3:	Test Substance Purity	High	× 1	1	
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable
	Metric 6:	Randomized Allocation	High	× 1	1	
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	
	Metric 12:	Exposure Route and Method	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Most test animal characteristics, including species, strain, sex, age, and starting body weight were reported; however, health status at the beginning of the study was not reported. The animals were obtained from a commercial source. The test species and strain were an appropriate animal model.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	
	Metric 15:	Number per Group	High	× 1	1	
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	

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Study Citation:	NTP (1990). Carcinogenesis studies of trichloroethylene (without epichlorohydrin) (CAS No. 79-01-6) in F344/N rats and B6C3F1 mice (gavage studies) Technical Report Series, 243
Data Type:	13-week oral (rats and mice)
HERO ID:	87574

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Food/water intake and respiratory rate were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	
	Metric 24:	Reporting of Data	High	× 2	2	
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 40: **Animal toxicity evaluation results of Maltoni et al 1986 for an 8-wk inhalation carcinogenicity study in rats and mice on cancer outcomes**

Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	8 week inhalation carcinogenicity study in rats and mice
HERO ID:	196223

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported, and it was stated that it was analyzed (batch number and other details not provided).
	Metric 3:	Test Substance Purity	Medium	× 1	2	The test substance was reportedly "highly purified;" percent purity was not reported. minor uncertainties regarding purity are not expected to impact the results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors reported using concurrent negative controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not required by study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The study indicated that animals were randomly distributed into groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The methods and equipment used to generate the test substance were reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were provided; it appears that animals were exposed consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Actual concentrations were not reported, but there is high confidence that animals were exposed at approximately the reported target concentrations. The study states that concentrations were checked by continuous gas-chromatograhic monitoring. Records of concentrations are presumably available (conserved in archives). CK: During the course of the treatment, the concentrations and distribution of TCE were checked by continuous gas-chromatographic monitoring.

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Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	8 week inhalation carcinogenicity study in rats and mice
HERO ID:	196223

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	Unacceptable	× 1	4	The study duration is not long enough to adequately assess carcinogenicity. The duration of exposure (8 weeks) differs significantly from typical study designs in rodents (i.e., 52 weeks or more). CK: Although different section of the study has 104 week exposure, this study is for 8 weeks and does not meet the criteria for a carcinogenicity study (tables 12 and 13)
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	There were minor limitations regarding concentration spacing (i.e. if the high dose was high enough). However, the lowest concentration was meant to coincide with the occupational limit of exposure for TCE (at least in some countries).
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic air chambers were used, providing 12-15 air changes/hour.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain, sex, and starting body weights (presented graphically in the Appendix) were provided. The study indicated that Sprague-Dawley rats and Swiss mice were "of the breed routinely employed in our Laboratories" (no further information provided), The choice of animals was intended to provide an integrated system of biological models and are the types of animals usually used in carcinogenicity assays. CK: changed from medium to high as the test animal species, strain and sex were adequately described and appropriate for evaluation of specific outcome
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were provided and were adequate and the same for exposed groups and controls.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported, and consistent with studies of the same type (i.e., >50/sex/group for carcinogenicity studies).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcome of interest (cancer); the timing of the assessment was at spontaneous death so that the potential for neoplastic effects could be evaluated.

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Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	8 week inhalation carcinogenicity study in rats and mice
HERO ID:	196223

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There were differences in the timing of the assessment (animals were allowed to live until spontaneous death), but the outcome assessment protocol was consistent across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals were subjected to histopathological examinations (> 35 tissues); carcinogenicity was assessed in an adequate number of animals/group.
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding is not required for initial histopathology review; however, the study indicated that slides were screened by a junior pathologist and reviewed by the same senior pathologist throughout the study.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control groups were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	There were no reported differences among study groups with respect to initial body weights. Lack of data on respiratory rates in not likely to impact the results.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	Data for animal attrition were provided (graphically) in the Appendix; there are not differences that would influence the outcome assessment.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were described and appropriate for the datasets of interest.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were reported for outcomes by exposure and sex.
Overall Quality Determination‡			~~Unacceptable~~** ⟶ Low§		~~1.4~~	
Extracted			Yes			

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Although the study is well-conducted, the duration of the study is too short to adequately assess carcinogenicity. CK: This is a large study including different exposure durations. This part of the study is a 8 week study which to too short to assess the carcinogenic potential. Hence, one metric (10) was rated unacceptable and so the overall judgement was unacceptable. This is a very well conducted study, and can be considered as a support study for the chronic exposure part of the study (carcinogenicity)"

Table 41: **Animal toxicity evaluation results of Arito et al 1994 for a 6-wk inhalation study on cardiovascular, neurological/behavior outcomes**

Study Citation:	Arito, H; Takahashi, M; Ishikawa, T (1994). Effect of subchronic inhalation exposure to low-level trichloroethylene on heart rate and wakefulness-sleep in freely moving rats Sangyo Igaku/Japanese Journal of Industrial Health (Japan), 36(1), 1-8
Data Type:	6-week inhalation
HERO ID:	61300

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE was not identified by CASRN, but the name is unambiguous
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was not reported. No other information was reported about TCE either.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity or grade of the test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate concurrent negative control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable - a positive control group is not indicated by the study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	There was no information reported on storage, but animals were exposed to TCE vapor which would naturally evaporate from a liquid reservoir of the substance. It appears that the vapor is simply airflow passed over TCE.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration (e.g., exposure frequency; same time of day; consistent chamber designs; animals/chamber) were reported and exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	TCE concentration was analytically measured based on the author's statement of less than 5% variation, but it is unclear whether this is nominal or analytical concentration. TCE is relatively stable but can react with light and water, with a half-life of 5 days in sunlight (NCBI). It is stable under recommended storage conditions (PubChem). Therefore, It is assumed that while storage of TCE may be in issue, the absence of information does not disqualify the study.

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Study Citation: Arito, H; Takahashi, M; Ishikawa, T (1994). Effect of subchronic inhalation exposure to low-level trichloroethylene on heart rate and wakefulness-sleep in freely moving rats Sangyo Igaku/Japanese Journal of Industrial Health (Japan), 36(1), 1-8
Data Type: 6-week inhalation
HERO ID: 61300

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and the outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups and concentration spacing were appropriate for the outcomes of interest. Although the concentrations were not specifically justified by the study authors, the concentrations were discussed in the context of a previous, shorter-term study (reference no. 11) and are appropriate. Ideally would have had a lower dose with no effect, as all doses show similar albeit dose-responsive effects.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route was reported but the method of exposure was not. The number of air changes per hour was acceptable (15 times/hour). While the method of exposure was not reported, I did not downgrade the score because TCE is not expected to condense to form an aerosol.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Most test animal characteristics (species, strain, sex, age) were reported; however, starting body weight and health status at the beginning of the study were not reported so I downgraded the score to medium.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported and were adequate and the same for the control and exposed groups.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per group (5 males/group) was lower than the typical number used in studies of a similar type but sufficient for statistical analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcomes and was sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., measurements performed at the same time during and after exposure).
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable - No evaluations that were considered subjective were conducted, so I considered this metric to be not applicable.

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Study Citation:	Arito, H; Takahashi, M; Ishikawa, T (1994). Effect of subchronic inhalation exposure to low-level trichloroethylene on heart rate and wakefulness-sleep in freely moving rats Sangyo Igaku/Japanese Journal of Industrial Health (Japan), 36(1), 1-8
Data Type:	6-week inhalation
HERO ID:	61300

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 20: Negative Control Response	High	× 1	1	The biological responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in test design and procedures were reported; however, respiratory rate, initial body weight, and food/water intake were not reported. TCE is a potential irritant and due to the lack of reporting of respiratory rate measurement, I downgraded the score to low.
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and health outcomes unrelated to exposure for each study group were not reported, so I downgraded the score to medium.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Statistical methods were described and appropriate for the datasets.
	Metric 24: Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group by way of figures, from which numerical data could be derived for re-analysis if necessary. Negative findings were reported qualitatively or quantitatively. The presentation of statistical significance for concentration vs exposure repetition was confusing and difficult to track, but the body text did clarify the data for all assays.
Overall Quality Determination‡		~~Medium~~ ⟶ Medium§		~~1.7~~	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Based on the uncertainty over the test substance storage and preparation, which could potentially greatly affect the reliability of measurements in the study over time"

Table 42: **Animal toxicity evaluation results of Gash et al 2008 for a 6-wk gavage neurotoxicity study in rats on neurological/behavior outcomes**

Study Citation:	Gash, D; Rutland, K; Hudson, N; Sullivan, P; Bing, G; Cass, W; Pandya, J; Liu, M; Choi, D; Hunter, R; Gerhardt, G; Smith, C; Slevin, J; Prince, T (2008). Trichloroethylene: Parkinsonism and complex 1 mitochondrial neurotoxicity Annals of Neurology, 63(2), 184-192
Data Type:	6 week gavage neurotoxicity study in rats
HERO ID:	700905

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance obtained from manufacturer with no details of lot number or identity verification.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and test grade were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham-treated negative controls given vehicle were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not typical for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not describe how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage was not reported.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Study did not report time of day when animals were dosed. Oral absorption of TCE may be affected by fasting, so time of administration is an important factor. Gavage volume was not reported but is expected to be within recommended values based on density of TCE and reported volume of vehicle.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Dose was reported in mg/kg but frequency of adjustment of dosing for body weight changes was not reported. The animals were 5 months old and thus would were not in growth phase, so body weight changes may not have been significant., but no information was presented regarding initial or final weight or changes over the exposure duration.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Exposure was 5 d/wk which is typical for gavage administration. 6 week duration is adequate for endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only one nonzero dose was used.

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Study Citation:	Gash, D; Rutland, K; Hudson, N; Sullivan, P; Bing, G; Cass, W; Pandya, J; Liu, M; Choi, D; Hunter, R; Gerhardt, G; Smith, C; Slevin, J; Prince, T (2008). Trichloroethylene: Parkinsonism and complex 1 mitochondrial neurotoxicity Annals of Neurology, 63(2), 184-192
Data Type:	6 week gavage neurotoxicity study in rats
HERO ID:	700905

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Animals were exposed by gavage but details of the administration method were not reported.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Source of the test animal was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Some details of husbandry conditions were missing (i.e., temperature, cages, number per cage), but authors report that their protocol was approved by their university Animal Care and Use committee. No deviations in husbandry conditions were reported.
	Metric 15:	Number per Group	High	× 1	1	There were 9 animals per treatment group for the mitochondrial studies and 17/group for the histopathology examinations.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Sensitive endpoints (histopathology of specific areas of brain; brain and liver mitochondrial respiration and enyzyme activity) were evaluated. Methods for these evaluations were given in detail or cited to another paper.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	No inconsistencies in outcome assessment were noted in the paper, but few details of the outcome assessment protocol execution were provided.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Not all exposed animals were evaluated for all endpoints due multiple preparation methods. Mitochondrial assessments used pooled samples, so interindividual variation could not be evaluated.
	Metric 19:	Blinding of Assessors	High	× 1	1	Study reports bistology examination by blinded assessors.
	Metric 20:	Negative Control Response	High	× 1	1	Control responses were reported and appeared to be acceptable.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	No information on food or water intake was reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	No animal attrition was described, but no information was provided regarding observations other than the measured endpoints.
Domain 7: Data Presentation and Analysis						

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Study Citation:	Gash, D; Rutland, K; Hudson, N; Sullivan, P; Bing, G; Cass, W; Pandya, J; Liu, M; Choi, D; Hunter, R; Gerhardt, G; Smith, C; Slevin, J; Prince, T (2008). Trichloroethylene: Parkinsonism and complex 1 mitochondrial neurotoxicity Annals of Neurology, 63(2), 184-192
Data Type:	6 week gavage neurotoxicity study in rats
HERO ID:	700905

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 23: Statistical Methods	Low	× 1	3	Statistical analysis consisted of Studen't t-test, which does not account for multiple comparisons.
	Metric 24: Reporting of Data	Medium	× 2	4	Continuous endpoints were reported quantitatively (graphically), but incidences of histopathology changes were not given.
Overall Quality Determination‡		~~Unacceptable~~** ⟶ Low§		~~2.1~~	
Extracted		No			

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "The only metric for which the study was unacceptable was Metric 7 (preparation and storage); otherwise the study was well-conducted. It was upgraded because it measures sensitive endpoints (effects on substantia nigra) in the rat corroborating effects reported in humans exposed occupationally."

Table 43: **Animal toxicity evaluation results of Dow et. al 2000 for a 3-month drinking water study in rats on clinical chemistry/biochemical, hematological and immune outcomes**

Study Citation:	Dow, J; Green, T (2000). Trichloroethylene induced vitamin B(12) and folate deficiency leads to increased formic acid excretion in the rat Toxicology, 146(2-3), 123-136
Data Type:	3-month drinking water study in rats - trichloroethanol
HERO ID:	701799

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	trichloroethanol (primary metabolite of TCE).
	Metric 2:	Test Substance Source	Medium	× 1	2	commercial source identified, but did not provide lot/batch numbers
	Metric 3:	Test Substance Purity	Low	× 1	3	purity and/or grade of test substances were not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Results indicate that a control was used, but characterization of the control group were not reported.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for this study
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	There are deficiencies in reporting of test substance preparation and storage
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	administered continuously in drinking water with water consumption monitored
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	For 3-month study Administered doses in drinking water were 0.5 and 1.0 g/l; however, the low dose was reduced to 0.35 g/l after 4 weeks. Authors calculated actual daily doses of 22.1 and 65.2 mg/kg-day based on water consumption for 0.35 and 1.0 g/l, respectively. The calculated dose for the concentration of 0.5 g/l was not calculated into units of mg/kg by the authors.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	administered doses in drinking water were 0.5 and 1.0 g/l; however, the dose spacing was altered 4 weeks into the study because a dose-response was not shown between the 2 treatment groups; the low dose was reduced to 0.35 g/l after 4 weeks.
	Metric 12:	Exposure Route and Method	High	× 1	1	

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Study Citation:	Dow, J; Green, T (2000). Trichloroethylene induced vitamin B(12) and folate deficiency leads to increased formic acid excretion in the rat Toxicology, 146(2-3), 123-136
Data Type:	3-month drinking water study in rats - trichloroethanol
HERO ID:	701799

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 4: Test Organism					
Metric 13:	Test Animal Characteristics	High	× 2	2	
Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Humidity and temperature were not reported, but noted to be in temperature controlled rooms
Metric 15:	Number per Group	Medium	× 1	2	5 rats/group
Domain 5: Outcome Assessment					
Metric 16:	Outcome Assessment Methodology	High	× 2	2	
Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
Metric 18:	Sampling Adequacy	High	× 1	1	
Metric 19:	Blinding of Assessors	Not Rated	NA	NA	
Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control					
Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	lack of reporting of water intake was not reported.
Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis					
Metric 23:	Statistical Methods	Low	× 1	3	statistical methods not clearly described
Metric 24:	Reporting of Data	Medium	× 2	4	
Overall Quality Determination‡		~~Medium~~ ⟶ Low§		~~1.9~~	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "CK: I am downgrading this study to low because there are several flaws in the study. The drinking water experiment is part of other experiments. Only 5 animals were used per group, Two doses were used (0.5 and 1.0g/l) but after 4 weeks of dosing the 0.5 g/l was reduced to 0.35g/l without specific reasoning. It is assumed that the control animals were given water as vehicle, but no description is given except the results show data for control animals. The exact purity of the compound was not provided. Also the endpoints evaluated were more mechanistic other than the formate in urine."

Table 44: **Animal toxicity evaluation results of Kaneko et al 2000 for a subchronic immunotoxicity study in mice on hematological, immune, body weight, hepatic, renal, and gastrointestinal outcomes**

Study Citation:	Kaneko, T; Saegusa, M; Tasaka, K; Sato, A (2000). Immunotoxicity of trichloroethylene: A study with MRL-lpr/lpr mice Journal of Applied Toxicology, 20(6), 471-475					
Data Type:	Immunotoxicity in MRL-lpr/lpr mice					
HERO ID:	706345					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE CK: Detailed Test Substance information provided
	Metric 2:	Test Substance Source	Medium	× 1	2	Wako Pure Chemical Industries; batch no. not reported CK: TCE (purity >99%, Wako Pure Chemical Industries, Osaka, Japan)
	Metric 3:	Test Substance Purity	High	× 1	1	>99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	air control
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not required by study design.
	Metric 6:	Randomized Allocation	High	× 1	1	animals were randomly divided into four groups CK: Five animals for each group
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	TCE was prepared by injecting liquid TCE into a metered air stream at a known rate by means of a power-driven syringe. The air stream was led into a preliminary chamber where the vapor was mixed thoroughly and then into the exposure chamber. This continuous aeration was to maintain the target concentration. Information on storage not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Consistent between groups. The atmospheric concentration was monitored with a gas chromatographic autosampling system every 20 min during exposure
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Only target concentrations were reported; however, The atmospheric concentration was monitored with a gas chromatographic autosampling system every 20 min during exposure and continuous aeration was used to maintain the target concentration
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	8 wks, 4 hr/d, 6 d/wk; 4 hr/d shorter than standard (6 hr/d)

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Study Citation:	Kaneko, T; Saegusa, M; Tasaka, K; Sato, A (2000). Immunotoxicity of trichloroethylene: A study with MRL-lpr/lpr mice Journal of Applied Toxicology, 20(6), 471-475
Data Type:	Immunotoxicity in MRL-lpr/lpr mice
HERO ID:	706345

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	3 exposed plus control.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Whole-body, dynamic exposure. Number of air changes/hour was not reported (nor was flow rate).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Study in mouse strain genetically susceptible to autoimmune diseases (MRL-lpr/lpr mice). No standard mouse strains evaluated in this study. CK: The test animal was appropriate for the evaluation of the specific outcome(s) of interest (e.g., genetically modified animals) Pneumatosis cystoides intestinalis is not a type of disease where a dose–response relationship with TCE exposure can be recognized and it is difficult to reproduce its physiopathology through TCE exposure in ordinary experimental animals. In the present study, immunological changes caused by TCE exposure were investigated by employing MRL-lpr/lpr mice that are genetically labile to autoimmune diseases.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Housing and room conditions reported. Food and water ad libitum except during exposure.
	Metric 15:	Number per Group	High	× 1	1	5 males/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Body weight, various immunological endpoints, histology of "immunoreactive" organs (thymus, liver, spleen, intestines, kidney).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	5 males/group
	Metric 19:	Blinding of Assessors	High	× 1	1	For histology, the assessor was blind. Other endpoints are quantitative (blinding not required).
	Metric 20:	Negative Control Response	High	× 1	1	Control responses reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	No changes in BW before or during exposure. Respiratory rate not reported, but since TCE produces little to no respiratory irritation at anesthetic doses, bradypnea is not expected to be an issue.

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Study Citation:	Kaneko, T; Saegusa, M; Tasaka, K; Sato, A (2000). Immunotoxicity of trichloroethylene: A study with MRL-lpr/lpr mice Journal of Applied Toxicology, 20(6), 471-475
Data Type:	Immunotoxicity in MRL-lpr/lpr mice
HERO ID:	706345

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Renal dysfunction was observed in all groups. study authors indicate that the mice used in this study are known to develop a renal dysfunction spontaneously around the age of 15 weeks, and the renal changes observed were not attributed to TCE exposure
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Student's t-tests were used for continuous data. No statistics reported for histopathological lesions; lesions only reported qualitatively.
	Metric 24:	Reporting of Data	Medium	× 2	4	Exposure-related histopathological changes only reported qualitatively. Immune endpoints with exposure-related findings reported graphically. Body weights reported graphically.
Overall Quality Determination‡			High		1.5	
Extracted			No			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 45: **Animal toxicity evaluation results of Kauffmann et al 1982 for a 14-day gavage study and 90-day drinking water study in mice on hematological and immune outcomes**

Study Citation:	Kauffmann, B; White, K; Sanders, V; Douglas, K; Sain, L; Borzelleca, J; Munson, A (1982). Humoral and cell-mediated immune status in mice exposed to chloral hydrate Environmental Health Perspectives, 44 147-151
Data Type:	14-day gavage study and 90-day drinking water studies in mice - immunotoxicity
HERO ID:	706374

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance is identified as chloral hydrate (metabolite of TCE)
	Metric 2:	Test Substance Source	High	× 1	1	The test substance source was not reported in this publication; however, it was reported in a related publication (Sanders et al., 1982). Source: U.S.P. crystalline, J. T. Baker Co., Phillipsburg, N.J. 08865, lot #925086)
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was not reported in this publication; however, it was reported in a related publication (Sanders et al., 1982). purity was 99+%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	deionized water
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for this study type
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how the animals were allocated to study groups; allocation was not reported in a related previously published study.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The study did not report about the preparation and storage, but was reported in a related publication ((Sanders et al., 1982); CK: chemical preparation was reported but not storage
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	The study did not report details about exposure administration to evaluate consistency; but was reported in a related publication (Sanders et al., 1982).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Gavage doses reported for 14-day study; for the drinking water studies, that actual doses of chloral hydrate consumed as time weighted averages were described in a related publication (Sanders et al., 1982).. The related publication reported TWA doses to be 18 and 173 mg/kg-day for females and 16 and 160 mg/kg-day in males.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	14 days and 30 day drinking water study and 14 day gavage study with two doses in each duration treatments

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Study Citation:	Kauffmann, B; White, K; Sanders, V; Douglas, K; Sain, L; Borzelleca, J; Munson, A (1982). Humoral and cell-mediated immune status in mice exposed to chloral hydrate Environmental Health Perspectives, 44 147-151
Data Type:	14-day gavage study and 90-day drinking water studies in mice - immunotoxicity
HERO ID:	706374

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	two drinking water exposure groups with doses 14.4 mg/kg or 144 mg/kg (14 day study) and 0.07mg/ml or 0.7mg/ml (3 months study)
	Metric 12:	Exposure Route and Method	High	× 1	1	gavage and drinking water
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The study reported some details about test animal characteristics; however, the strain, sex, and beginning weights were reported in the related publication. (Sanders et al., 1982). Animals were obtained from a commercial source.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	The study did not report details about animal husbandry conditions; however, it was reported in a related publication ((Sanders et al., 1982).
	Metric 15:	Number per Group	High	× 1	1	number of animals/dose group were reported in study and in related study (Sanders et al., 1982).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	body weight, organ (spleen) weight, and other immunological endpoints were assessed
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	no major inconsistencies were observed
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling for the outcome of interest was reported and described in results tables
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not rated or applicable for this study.
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the control groups were adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among the study groups noted in this study or related publication (Sanders et al., 1982).
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	health outcomes unrelated to exposure were reported in related publication (Sanders et al., 1982).
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Appropriate statistical evaluation was reported
	Metric 24:	Reporting of Data	High	× 2	2	Adequate data results was presented in tables
Overall Quality Determination[‡]			High		1.1	
Extracted			Yes			

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Study Citation:	Kauffmann, B; White, K; Sanders, V; Douglas, K; Sain, L; Borzelleca, J; Munson, A (1982). Humoral and cell-mediated immune status in mice exposed to chloral hydrate Environmental Health Perspectives, 44 147-151
Data Type:	14-day gavage study and 90-day drinking water studies in mice - immunotoxicity
HERO ID:	706374

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

5 Chronic (>90 days)

Table 46: **Animal toxicity evaluation results of Gilbert et al 2012 for a 12- and 17-wk drinking water study in mice on hematological and immune outcomes**

Study Citation:	Gilbert, K.M., Nelson, A.R., Cooney, C.A., Reisfeld, B., Blossom, S.J. (2012). Epigenetic alterations may regulate temporary reversal of CD4(+) T cell activation caused by trichloroethylene exposure Toxicological Sciences, 127(1), 169-178
Data Type:	12- and 17-week drinking water study in mice
HERO ID:	2127984

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE is clearly identified
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported, but batch/lot number was not; unlikely to have a substantial impact on results.
	Metric 3:	Test Substance Purity	High	× 1	1	> 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	water was used as the negative control
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for this study type
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report the method of allocation; the impact on results are unclear.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	There were some ommissions in the reporting of test substance preparation and storage. Freshly made TCE-containing drinking water was provided every 2-3 days.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	chemical administration in drinking water for either 12 or 17 weeks
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	drinking water concentrations were reported; water consumption data was not reported.. Administered doses were calculated by the study authors to be 3, 14, 64 mg/kg-day based on water consumption (corresponding to 0.02, 0.1, and 0.5 mg/ml)in the 12-week study
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	12 or 17 week exposure through drinking water
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	12 mice per group for the 12 week study with three exposure groups (0,0.02, 0.1 or 0.5 mg/ml) or 10 mice per group for 17 week study with two exposure groups (0.01 or 0.1 mg/ml TCE)
	Metric 12:	Exposure Route and Method	High	× 1	1	drinking water
Domain 4: Test Organism						

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Study Citation:	Gilbert, K.M., Nelson, A.R., Cooney, C.A., Reisfeld, B., Blossom, S.J. (2012). Epigenetic alterations may regulate temporary reversal of CD4(+) T cell activation caused by trichloroethylene exposure Toxicological Sciences, 127(1), 169-178
Data Type:	12- and 17-week drinking water study in mice
HERO ID:	2127984

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Mice were obtained from a commercial source and strain, sex, age and body weight were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported; impact on results are unclear.
	Metric 15:	Number per Group	High	× 1	1	10 or 12 mice/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	same across groups
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not rated/applicable for this study
	Metric 20:	Negative Control Response	High	× 1	1	water was used a negative control
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	no known confounding variables
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	no attrition was reported, doesn't seem to have an impact on the results
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Appropriate statistical methods were applied
	Metric 24:	Reporting of Data	High	× 2	2	Body weight and other mechanistic (immunological epigenetic alterations) were reported
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 47: **Animal toxicity evaluation results of Rohm Haas Co 1982 for a 2-yr exposure study in rats and mice on cancer and mortality outcomes**

Study Citation:	Rohm & Haas Co (1982). Initial submission: Carcinogenesis bioassay with trichloroethylene in rats and mice (draft report) with cover letter dated 080392
Data Type:	2-year oral cancer bioassay, rats, mice
HERO ID:	4215768

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name, CASRN, structure, formula, and mol. weight
	Metric 2:	Test Substance Source	High	× 1	1	The source and lot numbers were reported and identity analysis was conducted. The infrared and nuclear magnetic resonance spectra were reported to be consistent with literature spectra, although the data were not included in the pages of the document.
	Metric 3:	Test Substance Purity	High	× 1	1	The composition was such that effects likely due to test substance. The percentage of impurities and type of stabilizer used were identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Vehicle and untreated concurrent negative control groups were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control was not required for this assay.
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly assigned to cases and treatment group.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage information were reported and stability was tested.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	The volume received by rats and mice was administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Animals received the doses daily. CK: Animals were administered five times per week for 103 weeks,
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The dose groups were based on a 13-week study.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route and method were suited to the test substance based on reported results of the stability tests..
Domain 4: Test Organism						

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Study Citation:	Rohm & Haas Co (1982). Initial submission: Carcinogenesis bioassay with trichloroethylene in rats and mice (draft report) with cover letter dated 080392
Data Type:	2-year oral cancer bioassay, rats, mice
HERO ID:	4215768

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The source, strain, sex, age, and starting body weight were reported. The health status was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported and were appropriate.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was reported and appropriate for the study.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcomes methodology assessment addressed outcomes.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric was not required for endpoints in this study.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses were appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were not reported differences.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes unrelated to exposure were observed.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analyses were described for neoplastic outcomes, but limited information analysis of non-neoplastic outcomes was reported.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data on non-neoplastic lesions are in the non-attached appendices. Other data were reported.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 48: **Animal toxicity evaluation results of Henschler et al 1980 for a 18-month inhalation study in 3 species on cancer, mortality, and body weight outcomes**

Study Citation:	Henschler, D; Romen, W; Elsaesser, HM; Reichert, D; Eder, E; Radwan, Z (1980). Carcinogenicity study of trichloroethylene by longterm inhalation in three animal species Archives of Toxicology, 43(4), 237-248
Data Type:	18-month inhalation study - 3 species, cancer
HERO ID:	65250

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Pure trichloroethylene, stabilized by an amine base,
	Metric 2:	Test Substance Source	Medium	× 1	2	Source of test substance not reported, but analysis indicated that highly purified TCE, stabilized with 0.0015% triethanolamine, was used throughout. GC-MS analysis revealed the following impurities in the liquid: chloroform, carbon tetrachloride, l,l,2-trichloroethane, 1,1,1-trichloroethane, 1,1,2,2-tetrachloroethane, each being present to less than 0.000025% (w/w).
	Metric 3:	Test Substance Purity	High	× 1	1	GC-MS analysis revealed the following impurities in the liquid: chloroform, carbon tetrachloride, l,l,2-trichloroethane, 1,1,1-trichloroethane, 1,1,2,2-tetrachloroethane, each being present to less than 0.000025%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative controls were used. It is not clear if they were untreated or sham exposed (not downgraded here - down graded in Metric 8). Not reporting sham is technically a low according to the criteria, but since the only options for control were air or nitrogen diluted into air, this is unlikely to make a substantial difference.
	Metric 5:	Positive Controls	Not Rated	NA	NA	No necessary based on study design.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	For the preparation of TCE-air-mixtures, liquid TCE was continuously supplied by a motor-driven syringe for evaporation into a thermostatically heated glass column (30 cm x 30 mm i.D.), filled with glass beads. A stream of highly purified nitrogen carried the vaporized TCE into a mixing device for further dilution with air to the final concentration before entering the exposure chamber.

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Study Citation:	Henschler, D; Romen, W; Elsaesser, HM; Reichert, D; Eder, E; Radwan, Z (1980). Carcinogenicity study of trichloroethylene by longterm inhalation in three animal species Archives of Toxicology, 43(4), 237-248
Data Type:	18-month inhalation study - 3 species, cancer
HERO ID:	65250

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Unclear if controls were unexposed or sham-exposed. Exposure in exposed groups was consistent. The absence of reporting of sham controls was accounted for in metric 4.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Only target concentrations were reported. The exposure concentrations were monitored by direct UV spectrometry (Beckman Type 25) at 205 nm in quartz flow cells of I cm (500 ppm) or 5 cm (100 ppm) pathlength; but analytical concentrations not reported.
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	18 months (5 d/wk, 6 hr/d); but animals not sacrificed until 30 months (mice and hamsters) or 36 months (rats). While not the standard design due to long recovery period, it is not inappropriate for the endpoints in this form (cumulative mortality, body weight over the course of the study, tumors). Study authors were attempting to mimic occupational exposure of a majority of lifetime, but not entire lifetime. The authors are ambiguous as to whether there was truly a 12-18month recovery period prior to any dissection/autopsy or whether the majority of examinations occurred after 18mo of exposure. It cannot be assumed that there was not recovery period based on the wording, however. A recovery period is not ideal, however
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Two exposure levels plus control. No evidence of toxicity, but high dose considered high enough by study authors because it is 10-fold the maximal concentration in the workplace (50 ppm) at the time of the study.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Dynamic whole-body exposure chamber, only 3 air changes per hour
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain, sex, and source reported. Age and initial body weight not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Individual cages, consistent environmental conditions (rel. humidity 45-50%; temperature 23 ~ C; daylight rhythm 7 a.m./7 p.m.). A diet of standardized pellets and tap water was offered ad libitum from 2 p.m.--8 a.m. on exposure days, and without restrictions on weekends

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Study Citation:	Henschler, D; Romen, W; Elsaesser, HM; Reichert, D; Eder, E; Radwan, Z (1980). Carcinogenicity study of trichloroethylene by longterm inhalation in three animal species Archives of Toxicology, 43(4), 237-248
Data Type:	18-month inhalation study - 3 species, cancer
HERO ID:	65250

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	30/sex/group/ Less than the standard 50/sex/group, but adequate for analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Survival monitored throughout, body weight checked weekly. Only major, non-reproductive organs (spleen, liver, kidney, lung, heart, stomach, CNS tissue) plus all tissues with masses examined for tumors. The strain of mice used uniquely exhibits the lymphoma that appeared in the study, making the conclusions ambiguous and therefore being of questionable significance overall.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Consistent across groups. Data appears to be pooled from deceased and sacrificed animals, without any separate tracking. Negative data from animals that died early in the study could represent false negative data.
	Metric 18:	Sampling Adequacy	High	× 1	1	Examined in all dose groups; 30/sex/group.
	Metric 19:	Blinding of Assessors	High	× 1	1	2 independent, blinded histological assessors
	Metric 20:	Negative Control Response	Medium	× 1	2	Control data reported for tumors and survival. Control data for BW implied by "no changes in BW" statement. The negative controls routinely exhibited higher tumor numbers than the 100ppm exposure group, which should not be expected. It was also unclear whether the mortality data of controls was appropriate, with >50% of male mice dying prior to 2years.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	initial BW not reported; qualitative reporting of lack of BW effects. Respiratory rate not evaluated. However, TCE shows little to no respiratory irritation at anesthetic concentrations (HSDB).
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						

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Study Citation:	Henschler, D; Romen, W; Elsaesser, HM; Reichert, D; Eder, E; Radwan, Z (1980). Carcinogenicity study of trichloroethylene by longterm inhalation in three animal species Archives of Toxicology, 43(4), 237-248
Data Type:	18-month inhalation study - 3 species, cancer
HERO ID:	65250

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 23: Statistical Methods	Medium	× 1	2	Probability of survival and of observing tumors was estimated according to Kaplan and Meier (1958); comparison of survival of pairs of groups and age adjustment was performed using the methods of Cox (1972), Mantel (1963/66), Cochran (1954), and Saffiotti et al. (1972), following the description of the NCI study on trichloroethylene (1976). For comparison of tumor incidences, the chi-square test was applied. No statistics on BW, data reporting not adequate for independent analysis.
	Metric 24: Reporting of Data	Medium	× 2	4	Findings with exposure-related effects reported graphically or in tables. Findings without exposure-related effects reported qualitatively. The authors did not report the reason why there were one less male and two less female control animals. Tumor data was also not reported as a ratio or percentage.
Overall Quality Determination‡		~~Medium~~ ⟶ Low§		~~2.0~~	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Poor data reporting, non-ideal study design, limited organ examination, and questionable control values."

Table 49: **Animal toxicity evaluation results of Henschler et al 1980 for a 18-month inhalation study in 3 species on hepatic, renal, respiratory, cardiovascular, neurological/behavior, gastrointestinal, hematological, and immune outcomes**

Study Citation:	Henschler, D; Romen, W; Elsaesser, HM; Reichert, D; Eder, E; Radwan, Z (1980). Carcinogenicity study of trichloroethylene by longterm inhalation in three animal species Archives of Toxicology, 43(4), 237-248
Data Type:	18-month inhalation study - 3 species, nonneoplastic
HERO ID:	65250

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Pure trichloroethylene, stabilized by an amine base,
	Metric 2:	Test Substance Source	Medium	× 1	2	Source of test substance not reported, but analysis indicated that highly purified TCE, stabilized with 0.0015% triethanolamine, was used throughout. GC-MS analysis revealed the following impurities in the liquid: chloroform, carbon tetrachloride, l,l,2-trichloroethane, 1,1,1-trichloroethane, 1,1,2,2-tetrachloroethane, each being present to less than 0.000025% (w/w).
	Metric 3:	Test Substance Purity	High	× 1	1	GC-MS analysis revealed the following impurities in the liquid: chloroform, carbon tetrachloride, l,l,2-trichloroethane, 1,1,1-trichloroethane, 1,1,2,2-tetrachloroethane, each being present to less than 0.000025%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative controls were used. It is not clear if they were untreated or sham exposed (not downgraded here - down graded in Metric 8). Not reporting sham is technically a low according to the criteria, but since the only options for control were air or nitrogen diluted into air, this is unlikely to make a substantial difference.
	Metric 5:	Positive Controls	Not Rated	NA	NA	No necessary based on study design.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	For the preparation of TCE-air-mixtures, liquid TCE was continuously supplied by a motor-driven syringe for evaporation into a thermostatically heated glass column (30 cm x 30 mm i.D.), filled with glass beads. A stream of highly purified nitrogen carried the vaporized TCE into a mixing device for further dilution with air to the final concentration before entering the exposure chamber.

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Study Citation:	Henschler, D; Romen, W; Elsaesser, HM; Reichert, D; Eder, E; Radwan, Z (1980). Carcinogenicity study of trichloroethylene by longterm inhalation in three animal species Archives of Toxicology, 43(4), 237-248
Data Type:	18-month inhalation study - 3 species, nonneoplastic
HERO ID:	65250

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Unclear if controls were unexposed or sham-exposed. Exposure in exposed groups was consistent. The absence of reporting of sham controls was accounted for in metric 4.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Only target concentrations were reported. The exposure concentrations were monitored by direct UV spectrometry (Beckman Type 25) at 205 nm in quartz flow cells of I cm (500 ppm) or 5 cm (100 ppm) pathlength; but analytical concentrations not reported.
	Metric 10:	Exposure Frequency and Duration	Unacceptable	× 1	4	18 months (5 d/wk, 6 hr/d); but animals not sacrificed until 30 months (mice and hamsters) or 36 months (rats). This is not an appropriate design for evaluating organ weights or potentially reversible nonneoplastic lesions due to extended recovery period. The authors are ambiguous as to whether there was truly a 12-18month recovery period prior to any dissection/autopsy or whether the majority of examinations occurred after 18mo of exposure. It cannot be assumed that there was not recovery period based on the wording, however. For noncancer endpoints, this recovery period is unacceptable.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Two exposure levels plus control. No evidence of toxicity, but high dose considered high enough by study authors because it is 10-fold the maximal concentration in the workplace (50 ppm) at the time of the study.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Dynamic whole-body exposure chamber, only 3 air changes per hour
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain, sex, and source reported. Age and initial body weight not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Individual cages, consistent environmental conditions (rel. humidity 45-50%; temperature 23 ~ C; daylight rhythm 7 a.m./7 p.m.). A diet of standardized pellets and tap water was offered ad libitum from 2 p.m.--8 a.m. on exposure days, and without restrictions on weekends
	Metric 15:	Number per Group	Medium	× 1	2	30/sex/group/ Less than the standard 50/sex/group, but adequate for analysis.

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Study Citation:	Henschler, D; Romen, W; Elsaesser, HM; Reichert, D; Eder, E; Radwan, Z (1980). Carcinogenicity study of trichloroethylene by longterm inhalation in three animal species Archives of Toxicology, 43(4), 237-248
Data Type:	18-month inhalation study - 3 species, nonneoplastic
HERO ID:	65250

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Evaluated after recovery period Organ weights: spleen, liver, kidney, lung, heart Nonneoplastic histo: spleen, liver, kidney, lung, heart, stomach, CNS tissue. Evaluations were also made from deceased animals as they died throughout the study. Nonneoplastic lesions and other histopathological observations were not reported, along with changes in body weight and other toxicological endpoints as recommended by PECD 451.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Consistent across groups. Data appears to be pooled from deceased and sacrificed animals, without any separate tracking. Negative data from animals that died early in the study could represent false negative data. Downgraded to medium.
	Metric 18:	Sampling Adequacy	High	× 1	1	Examined in all dose groups; 30/sex/group.
	Metric 19:	Blinding of Assessors	High	× 1	1	2 independent, blinded histological assessors
	Metric 20:	Negative Control Response	Medium	× 1	2	Only nonneoplastic finding reported for exposed groups was also reported for control (liver cysts in hamsters) OW data not reported for any group.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	initial BW not reported; qualitative reporting of lack of BW effects. Respiratory rate not evaluated. However, TCE shows little to no respiratory irritation at anesthetic concentrations (HSDB).
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	No statistics conducted for organ weight or nonneoplastic lesions. No organ weight data reported. Only nonneoplastic lesion reported (liver cysts in hamsters) is adequate for independent analysis.
	Metric 24:	Reporting of Data	Unacceptable	× 2	8	Organ weight data not reported for any group. The only nonneoplastic finding reported was liver cysts in hamsters. No statement indicating that no other nonneoplastic findings were observed in hamsters,. No statement regarding nonneoplastic findings in mice or rats.

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Study Citation:	Henschler, D; Romen, W; Elsaesser, HM; Reichert, D; Eder, E; Radwan, Z (1980). Carcinogenicity study of trichloroethylene by longterm inhalation in three animal species Archives of Toxicology, 43(4), 237-248
Data Type:	18-month inhalation study - 3 species, nonneoplastic
HERO ID:	65250

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		Unacceptable⋆⋆		2.1	
Extracted		No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 50: **Animal toxicity evaluation results of Kjellstrand et al 1983 for a 30- or 120-day inhalation, intermittent study on hepatic, renal, hematological and immune, and body weight outcomes**

Study Citation:	Kjellstrand, P; Holmquist, B; Alm, P; Kanje, M; Romare, S; Jonsson, I; Månsson, L; Bjerkemo, M (1983). Trichloroethylene: Further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice Acta Pharmacologica et Toxicologica, 53(5), 375-384
Data Type:	30 or 120d inhalation, intermittent
HERO ID:	65255

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Commercial trichloroethylene, stabilized with 0.01 % thymol and 0.03 % diisopropylamine
	Metric 2:	Test Substance Source	High	× 1	1	Manufactured by Billerud-Uddeholm AB, Skoghall, Sweden. Batch number not reported, independent identity analysis not reported. Batch number not needed since TCE does not vary in composition.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Not reported, but identified as commercial grade
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent air-exposed controls were used for each test group.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed for study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Details regarding generation of the test substance were not given, but a prior publication was cited for exposure details (Kjellstrand et al. 1981) and may contain those details. Stock concentration (assumed 100%) was not reported, and the exposure system was not thoroughly described. The authors mentioned stabilizers mixed in with TCE.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Different exposure groups were exposed for different durations (shorter daily durations for higher concentrations), but each group had its own concurrent control. Exposure levels dropped in all exposure groups twice weekly when chambers were opened for changing of bedding, water and food.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Only target concentrations were reported. Study authors indicate that "In principle it is a dynamic system with an approximate +/-5 % range of random fluctuation at the highest concentration used and +/- 10% at the lowest"; however, analytical analysis of exposure levels were not reported.

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Study Citation:	Kjellstrand, P; Holmquist, B; Alm, P; Kanje, M; Romare, S; Jonsson, I; Månsson, L; Bjerkemo, M (1983). Trichloroethylene: Further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice Acta Pharmacologica et Toxicologica, 53(5), 375-384
Data Type:	30 or 120d inhalation, intermittent
HERO ID:	65255

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	Intermittent exposure for 30 or 120 d; recovery groups exposed for 30 days and held for 1 d recovery period or 120 d and held for 30d recovery period. Daily durations were different between groups. 16 hr/d for 225 ppm, 8 hr/d for 450 ppm, 4 hr/d for 900 ppm, 2 hr/d for 1800 ppm, and 1 hr/d for 3600 ppm. The shorter-durations at higher exposures differ significantly from typical study designs. While each group had its own control, makes comparison across groups challenging.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	For the 30 d study, there were 5 exposure groups plus control. For the 120 d study, the 120 d study plus 30 d recovery and 30 d study plus 1 d recovery there was only 1 exposure group plus control (PECO requires 2).
	Metric 12:	Exposure Route and Method	Low	× 1	3	A prior publication was cited for exposure details (Kjellstrand et al. 1981) . Only details in this report are that it was a dynamic, whole-body exposure chamber. Neither this or the 1981 publication provides any information on air changes or chamber size.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	White male and female NMRI mice (Anticimex, Sweden). Initial BWs in Table 2. Age not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	The animals were separated by sex and housed in groups of ten in transparent cages. Commercial laboratory mouse food (AB Astra Evos, Sweden) and water were freely available. The temperature was 22+-2". A 12 hour lighting schedule was controlled automatically with half an hour of twilight at dawn and dusk. Humidity not reported.
	Metric 15:	Number per Group	High	× 1	1	10-20/sex/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Hepatic: OW and histo, blood BuChE activity [HIGH] Spleen, Kidney: OW only [MEDIUM] Body weight [HIGH] Rated above based on the lowest rating per organ/organ system [MEDIUM]

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Study Citation:	Kjellstrand, P; Holmquist, B; Alm, P; Kanje, M; Romare, S; Jonsson, I; Månsson, L; Bjerkemo, M (1983). Trichloroethylene: Further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice Acta Pharmacologica et Toxicologica, 53(5), 375-384
Data Type:	30 or 120d inhalation, intermittent
HERO ID:	65255

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation across study groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	10-20/sex/group. Information not provided on technical replicates for BuChE activity. Downgraded to medium
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not needed for endpoints assessed.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group(s) were adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial BW comparable between groups. Some groups showed decreased BWs, but values showed <20% difference from control. Respiratory rate not evaluated, but TCE causes little or no irritation to the respiratory tract at anesthetic concentrations (HSDB).
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	t-test for continuous data; liver histological data was not analyzed statistically and data were reported only qualitatively.
	Metric 24:	Reporting of Data	Medium	× 2	4	Body weight, organ weight, and BuChE reported quantitatively. Liver histology findings reported quantitatively only.
Overall Quality Determination‡			Medium		1.9	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 51: **Animal toxicity evaluation results of Kjellstrand et al 1983 for a 30- or 120-day inhalation, continuous study on hepatic, renal, hematological and immune, and body weight outcomes**

Study Citation:	Kjellstrand, P; Holmquist, B; Alm, P; Kanje, M; Romare, S; Jonsson, I; Månsson, L; Bjerkemo, M (1983). Trichloroethylene: Further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice Acta Pharmacologica et Toxicologica, 53(5), 375-384
Data Type:	30 or 120d inhalation, continuous
HERO ID:	65255

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Commercial trichloroethylene, stabilized with 0.01 % thymol and 0.03 % diisopropylamine
	Metric 2:	Test Substance Source	High	× 1	1	Manufactured by Billerud-Uddeholm AB, Skoghall, Sweden. Batch number not reported, independent identity analysis not reported. Batch number not needed since TCE does not vary in composition.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Not reported, but identified as commercial grade
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent air-exposed controls were used for each test group.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed for study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Details regarding generation of the test substance were not given, but a prior publication was cited for exposure details (Kjellstrand et al. 1981) and may contain those details. Stock concentration (assumed 100%) was not reported, and the exposure system was not thoroughly described. The authors mentioned stabilizers mixed in with TCE.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Exposure was consistent across study groups. Exposure levels dropped in all exposure groups twice weekly when chambers were opened for changing of bedding, water and food.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Only target concentrations were reported. Study authors indicate that "In principle it is a dynamic system with an approximate +/-5 % range of random fluctuation at the highest concentration used and +/- 10% at the lowest"; however, analytical analysis of exposure levels were not reported.

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Study Citation:	Kjellstrand, P; Holmquist, B; Alm, P; Kanje, M; Romare, S; Jonsson, I; Månsson, L; Bjerkemo, M (1983). Trichloroethylene: Further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice Acta Pharmacologica et Toxicologica, 53(5), 375-384
Data Type:	30 or 120d inhalation, continuous
HERO ID:	65255

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Continuous exposure for 30 or 120 d; one group exposed for 30 days and held for 120 d recovery period. Continuous exposure isn't the standard design, but it is not a limitation.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	For the 30 d study, there were 3 exposure groups plus control. For the 120 d study and the 30 d plus 120 d recovery study, there was only 1 exposure group plus control (PECO requires 2).
	Metric 12:	Exposure Route and Method	Low	× 1	3	A prior publication was cited for exposure details (Kjellstrand et al. 1981) . Only details in this report are that it was a dynamic, whole-body exposure chamber. Neither this or the 1981 publication provides any information on air changes or chamber size.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	White male and female NMRI mice (Anticimex, Sweden). Initial BWs in Table 2. Age not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	The animals were separated by sex and housed in groups of ten in transparent cages. Commercial laboratory mouse food (AB Astra Evos, Sweden) and water were freely available. The temperature was 22+/-2". A 12 hour lighting schedule was controlled automatically with half an hour of twilight at dawn and dusk. Humidity not reported.
	Metric 15:	Number per Group	High	× 1	1	10/sex/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Hepatic: OW and histo, blood BuChE activity [HIGH] Spleen, Kidney: OW only [MEDIUM] Body weight [HIGH] Rated above based on the lowest rating per organ/organ system [MEDIUM]
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation across study groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	10/sex/group. Information not provided on technical replicates for BuChE activity. Downgraded to medium
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not needed for endpoints assessed.

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Study Citation:	Kjellstrand, P; Holmquist, B; Alm, P; Kanje, M; Romare, S; Jonsson, I; Månsson, L; Bjerkemo, M (1983). Trichloroethylene: Further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice Acta Pharmacologica et Toxicologica, 53(5), 375-384
Data Type:	30 or 120d inhalation, continuous
HERO ID:	65255

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group(s) were adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial BW comparable between groups. Some groups showed decreased BWs, but values showed <20% difference from control. Respiratory rate not evaluated, but TCE causes little or no irritation to the respiratory tract at anesthetic concentrations (HSDB).
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	t-test for continuous data; liver histological data was not analyzed statistically and data were reported only qualitatively.
	Metric 24:	Reporting of Data	Medium	× 2	4	Body weight, organ weight, and BuChE reported quantitatively. Liver histology findings reported quantitatively only.
Overall Quality Determination‡			Medium		1.8	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 52: **Animal toxicity evaluation results of NTP 1988 for a 2-yr cancer bioassay study on mortality, nutrition and metabolic/adult exposure body weight, hepatic, renal, respiratory, reproductive, hematological and immune, endocrine, neurological/behavior, cardiovascular, skin and connective tissue, thyroid, and gastrointestinal outcomes**

Study Citation:	NTP (1988). Toxicology and carcinogenesis studies of trichloroethylene (CAS No. 79-01-6) in four strains of rats (ACI, August, Marshall, Osborne-Mendel) (gavage studies)
Data Type:	2-year cancer bioassay
HERO ID:	65268

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance and lot numbers were reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was reported and sufficiently high such that the study results were likely to be due to the test substance itself. Impurities totaled less than 0.04%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate control group.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control is not indicated by the study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The study authors reported that animals were randomly allocated into study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation and storage conditions were reported and appropriate for the test substance.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were reported and methods were consistent among the groups. 14% of stock doses differed by more than 10% from target concentration.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for the study type and outcomes of interest. The test substance was administered orally 5 days/week for 103 weeks.

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Study Citation: NTP (1988). Toxicology and carcinogenesis studies of trichloroethylene (CAS No. 79-01-6) in four strains of rats (ACI, August, Marshall, Osborne-Mendel) (gavage studies)
Data Type: 2-year cancer bioassay
HERO ID: 65268

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	The number of dose groups and dose spacing were considered adequate to address the purpose of the study. The 13-week study was used to set dose levels for the 2-year study (discussed under Dose Selection Rational on p. 36). While the dose selection was based on the 13 week study, the doses for the 2-year study ended up being way too high, resulting in mortality and confounding kidney toxicity. Included peer reviewer comments mention that the study is probably not usable for kidney cancer based on this data. There are also only two exposure groups, while at least one more would be preferable for a non-cancer dose-response.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure (oral, gavage) were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal source, species, strain, sex, age, and starting body weight were reported. Health status was evaluated at the beginning of the study. The test animals were an appropriate animal model for evaluation of the specified outcomes of interest. Health status initially cannot be found, but health was monitored throughout, and they can be assumed to be healthy, and they were controlled for microflora.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported, including temperature, humidity, light-dark cycle) and were adequate and the same for the control and exposed populations.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group (50/sex/group) was reported and appropriate for the study type and outcome analysis and consistent with studies of the same or similar type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the outcomes of interest and was sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups using the same protocol in all study groups.

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Study Citation:	NTP (1988). Toxicology and carcinogenesis studies of trichloroethylene (CAS No. 79-01-6) in four strains of rats (ACI, August, Marshall, Osborne-Mendel) (gavage studies)
Data Type:	2-year cancer bioassay
HERO ID:	65268

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported and the study used adequate sampling for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were reported and histopathology was not described as re-evaluation so this metric is considered not applicable.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among the study groups in initial body weight. While food and water intakes were not reported, this is not expected to have a significant impact on the results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for the datasets.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data for exposure-related findings were presented for all outcomes. Negative findings were presented qualitatively and/or quantitatively. Data audit stated in the study found insufficient documentation of clinical observations, environmental conditions, and analytical chemistry data. Individual animal identification was not always verifiable. Downgraded to medium.
Overall Quality Determination‡			~~High~~ ⟶ Medium§		~~1.2~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "While no effects were observed at similar doses in the 13-week study, the two doses uses in this 2-year study were clearly too high, as high mortality and kidney toxicity was observed. The study was concluded to be inadequate for studying carcinogenic activity, and additional dose groups would be preferable for use of the noncancer endpoints, as extrapolation to lower doses may be inappropriate.."

Table 53: **Animal toxicity evaluation results of NCI 1976 for a 2-yr oral gavage exposure study in rats on cancer outcomes**

Study Citation:	NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type:	Cancer bioassay, gavage, rats
HERO ID:	75178

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained from manufacturer and identified by lot number and date of receipt.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity verified by GC and infrared spectroscopy to be at least 99%; impurities identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A sham-exposed negative control group received vehicle only.
	Metric 5:	Positive Controls	High	× 1	1	A concurrent positive control (carbon tetrachloride) was used.
	Metric 6:	Randomized Allocation	Medium	× 1	2	Study reports that "Animals were randomly1 assigned to treatment groups, so that initially the average weight in each group was approximately the same." and that "Animals were not distributed according to a table of random numbers."
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation and storage conditions were reported and appropriate for the test substance.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Details of exposure administration were reported, but deficiencies in administration of exposures (e.g., exposed at different times of day) are likely to have a substantial impact on results. The authors report that " In order to maintain the animals at the maximum doses that could be actually tolerated, body weight changes and survival were monitored, and, accordingly, doses were changed for the rats after 7 and 16 weeks of treatment, and for the mice after 12 weeks.To help assure survival until planned termination the dosing schedule was changed for rats to a cycle of 1 week of no treatment followed by 4 weeks of treatment."

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Study Citation: NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type: Cancer bioassay, gavage, rats
HERO ID: 75178

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Study reports that "All animals of one sex within a treatment group received the same dosage, that is, the volume of trichloroethylene solution administered to all animals was based on the mean body weight for the group." But since dosing volume may have varied within a group if body weights varied. Minor uncertainties in reporting of administered dose are unlikely to have a substantial impact on results.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Minor limitations in exposure frequency and duration of exposure were identified, but are unlikely to have a substantial impact on results. Animals were exposed 5 d/wk for 78 wks and then untreated for 32 additional weeks. The duration is suboptimal for a cancer bioassay (<2 yrs).
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose/concentration spacing were justified by study authors and considered adequate to address the purpose of the study.
	Metric 12:	Exposure Route and Method	High	× 1	1	Gavage administration was reported and appropriate to the study type.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animals were appropriate and obtained from commercial source, and strain, age, sex, body weight, and age at initiation of study were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were reported in detail and no deviations were reported. The animals were housed in the same room with animals used in other experiments, but this is not expected to affect the results because both control and exposed groups were housed in the same room.
	Metric 15:	Number per Group	Medium	× 1	2	There were minor limitations in the number of animals used for controls and the concerns are unlikely to have a substantial impact on results. Two doses of TCE was administered to 50 animals per sex; authors used 20 matched vehicle-treated controls for each sex and 99 male and 98 female rats vehicle-treated "colony controls".
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Rats were observed untreated for 32 weeks after the end of treatment, during which time tumor regression could have occurred.

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Study Citation: NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type: Cancer bioassay, gavage, rats
HERO ID: 75178

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	There were many deaths prior to scheduled termination, especially in the exposed groups (despite low apparent tumor incidences), so histopathology evaluations occurred after varying exposure durations.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals (except 2 low dose female rats described as "missing") were subjected to histopathology examination.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not considered critical for initial histopathology review.
	Metric 20:	Negative Control Response	Unacceptable	× 1	4	There was significant early mortality in control animals.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	The animals were housed in the same room with animals used in other experiments, but this is not expected to affect the results because both control and exposed groups were housed in the same room.
	Metric 22:	Health Outcomes Unrelated to Exposure	Unacceptable	× 1	4	There was significant mortality unrelated to exposure in all (including control) groups. Survival to scheduled sacrifice was low (12/40 controls; 21/100 low dose, and 16/100 high dose rats). The mortality was "generally" dose-related, but cause(s) of death was not established.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was reported and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Cancer data were reported at both group and individual pathology levels.
Overall Quality Determination‡			Unacceptable⋆⋆		1.6	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 54: **Animal toxicity evaluation results of NCI 1976 for a 2-yr gavage study in rats on renal and hepatic outcomes**

Study Citation:	NCI (1976). Carcinogenesis bioassay of trichloroethylene					
Data Type:	2 yr gavage study in rats					
HERO ID:	75178					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test material identified by unambiguous name
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained from manufacturer and identified by lot number and date of receipt
	Metric 3:	Test Substance Purity	High	× 1	1	Purity verified by GC and infrared spectroscopy to be at least 99%; impurities identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A sham-exposed negative control group received vehicle only.
	Metric 5:	Positive Controls	High	× 1	1	Positive control group was exposed to carbon tetrachloride
	Metric 6:	Randomized Allocation	Medium	× 1	2	Study reports that the animals were allocated randomly (albeit not by random number table) such that initial average weight of each group was approximately equal.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Details of preparation and storage were reported and should have been adequate to preserve TCE stability (e.g., prepared weekly, and stored in sealed and refrigerated containers), but stability was not tested.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	There were dose changes over time in both the low and high dose groups. In addition, for the last 48 weeks of treatment, the animals were exposed on a schedule of 4 weeks on and 1 week off.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Gavage volumes were not reported. In addition, dosing volume was determined by group mean body weight, so individual doses may have varied within a group if body weights varied.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Animals were exposed 5 d/wk for 78 wks and then observed untreated for 32 additional weeks. The duration is suboptimal for a cancer bioassay (<2 yrs)
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only two nonzero dose groups were included, and these differed by 2-fold. In addition, significant toxicity lead to a dose decrease after an earlier dose increase.
	Metric 12:	Exposure Route and Method	High	× 1	1	Gavage administration was reported and appropriate to the study type.

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Study Citation: NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type: 2 yr gavage study in rats
HERO ID: 75178

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animals were appropriate and obtained from commercial source, and strain, age, sex, BW, and age at initiation of study were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were reported in detail and no deviations were reported. The animals were housed in the same room with animals used in other experiments, but this is not expected to affect the results because both control and exposed groups were housed in the same room.
	Metric 15:	Number per Group	Medium	× 1	2	Dosed group sizes were 50/sex/dose, but only 20/sex for controls.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Rats were observed untreated for 32 weeks after the end of treatment, during which time regression of noncancer lesions could have occurred.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	There were many deaths prior to scheduled termination, especially in the exposed groups (despite low apparent tumor incidences), so histopathology evaluations occurred after varying exposure durations.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals (except 2 low dose female rats described as "missing") were subjected to histopathology examination.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not considered critical for initial histopathology review.
	Metric 20:	Negative Control Response	Low	× 1	3	There was significant early mortality in control animals.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	The animals were housed in the same room with animals used in other experiments, but this is not expected to affect the results because both control and exposed groups were housed in the same room.
	Metric 22:	Health Outcomes Unrelated to Exposure	Unacceptable	× 1	4	There was significant mortality unrelated to exposure in all (including control) groups. Survival to scheduled sacrifice was low (12/40 controls; 21/100 low dose, and 16/100 high dose rats). The mortality was "generally" dose-related, but cause(s) of death was not established. Authors noted a high incidence of chronic respiratory disease that did not differ across groups or sexes.
Domain 7: Data Presentation and Analysis						

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Study Citation: NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type: 2 yr gavage study in rats
HERO ID: 75178

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analysis was not performed for noncancer endpoints, but incidence data for independent statistical analysis are available in the individual animal pathology tables.
	Metric 24:	Reporting of Data	Low	× 2	6	incidence data are available in the individual animal pathology tables; however, severity data for chronic nephropathy were not reported on either group or individual basis.
Overall Quality Determination‡			Unacceptable⋆⋆		1.9	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 55: **Animal toxicity evaluation results of NCI 1976 for a 2-yr gavage study in mice on hepatic outcomes**

Study Citation:	NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type:	2 yr gavage study in mice
HERO ID:	75178

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test material identified by unambiguous name
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained from manufacturer and identified by lot number and date of receipt
	Metric 3:	Test Substance Purity	High	× 1	1	Purity verified by GC and infrared spectroscopy to be at least 99%; impurities identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A sham-exposed negative control group received vehicle only.
	Metric 5:	Positive Controls	High	× 1	1	Positive control group was exposed to carbon tetrachloride
	Metric 6:	Randomized Allocation	Medium	× 1	2	Study reports that the animals were allocated randomly (albeit not by random number table) such that initial average weight of each group was approximately equal.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Details of preparation and storage were reported and should have been adequate to preserve TCE stability (e.g., prepared weekly, and stored in sealed and refrigerated containers), but stability was not tested.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	There were dose changes over time in both the low and high dose groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Gavage volumes were not reported. In addition, dosing volume was determined by group mean body weight, so individual doses may have varied within a group if body weights varied.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Animals were exposed 5 d/wk for 78 wks and then observed untreated for 12 additional weeks.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only two nonzero dose groups were included, and these differed by ~2-fold. In addition, apparent lack of toxicity lead to a dose increase in both exposure groups
	Metric 12:	Exposure Route and Method	High	× 1	1	Gavage administration was reported and appropriate to the study type.
Domain 4: Test Organism						

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Study Citation: NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type: 2 yr gavage study in mice
HERO ID: 75178

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animals were appropriate and obtained from commercial source, and strain, age, sex, BW, and age at initiation of study were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were reported in detail and no deviations were reported. The animals were housed in the same room with animals used in other experiments, but this is not expected to affect the results because both control and exposed groups were housed in the same room.
	Metric 15:	Number per Group	Medium	× 1	2	Dosed group sizes were 50/sex/dose, but only 20/sex for controls.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Mice were observed untreated for 12 weeks after the end of treatment, during which time regression of noncancer lesions could have occurred.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There were no apparent variations across groups in outcome assessment. Premature deaths, especially of male mice (from liver tumors) lead to variations in the timing of histopathology evaluations.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals were subjected to histopathology examination.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not considered critical for initial histopathology review.
	Metric 20:	Negative Control Response	Medium	× 1	2	There was significant early mortality in control males (12/20).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	The animals were housed in the same room with animals used in other experiments, but this is not expected to affect the results because both control and exposed groups were housed in the same room.
	Metric 22:	Health Outcomes Unrelated to Exposure	Unacceptable	× 1	4	There was significant mortality, especially in male mice, likely related to liver tumors. Both the mortality and the tumors confound the interpretation of noncancer hepatic effects.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analysis was not performed for noncancer endpoints, but incidence data for independent statistical analysis are available in the individual animal pathology tables.

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Study Citation: NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type: 2 yr gavage study in mice
HERO ID: 75178

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	Low	× 2	6	incidence data are available in the individual animal pathology tables; however, severity data were not reported on either group or individual basis.
Overall Quality Determination‡		Unacceptable⋆⋆		1.8	
Extracted		No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 56: **Animal toxicity evaluation results of NCI 1976 for a 2-yr gavage study in mice on renal outcomes**

Study Citation:	NCI (1976). Carcinogenesis bioassay of trichloroethylene					
Data Type:	2 yr gavage study in mice					
HERO ID:	75178					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test material identified by unambiguous name
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained from manufacturer and identified by lot number and date of receipt
	Metric 3:	Test Substance Purity	High	× 1	1	Purity verified by GC and infrared spectroscopy to be at least 99%; impurities identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A sham-exposed negative control group received vehicle only.
	Metric 5:	Positive Controls	High	× 1	1	Positive control group was exposed to carbon tetrachloride
	Metric 6:	Randomized Allocation	Medium	× 1	2	Study reports that the animals were allocated randomly (albeit not by random number table) such that initial average weight of each group was approximately equal.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Details of preparation and storage were reported and should have been adequate to preserve TCE stability (e.g., prepared weekly, and stored in sealed and refrigerated containers), but stability was not tested.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	There were dose changes over time in both the low and high dose groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Gavage volumes were not reported. In addition, dosing volume was determined by group mean body weight, so individual doses may have varied within a group if body weights varied.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Animals were exposed 5 d/wk for 78 wks and then observed untreated for 12 additional weeks.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only two nonzero dose groups were included, and these differed by ~2-fold. In addition, apparent lack of toxicity lead to a dose increase in both exposure groups
	Metric 12:	Exposure Route and Method	High	× 1	1	Gavage administration was reported and appropriate to the study type.
Domain 4: Test Organism						

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Study Citation: NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type: 2 yr gavage study in mice
HERO ID: 75178

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animals were appropriate and obtained from commercial source, and strain, age, sex, BW, and age at initiation of study were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were reported in detail and no deviations were reported. The animals were housed in the same room with animals used in other experiments, but this is not expected to affect the results because both control and exposed groups were housed in the same room.
	Metric 15:	Number per Group	Medium	× 1	2	Dosed group sizes were 50/sex/dose, but only 20/sex for controls.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Mice were observed untreated for 12 weeks after the end of treatment, during which time regression of noncancer lesions could have occurred.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There were no apparent variations across groups in outcome assessment. Premature deaths, especially of male mice (from liver tumors) lead to variations in the timing of histopathology evaluations.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals were subjected to histopathology examination.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not considered critical for initial histopathology review.
	Metric 20:	Negative Control Response	Medium	× 1	2	There was significant early mortality in control males (12/20).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	The animals were housed in the same room with animals used in other experiments, but this is not expected to affect the results because both control and exposed groups were housed in the same room.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	There was significant mortality, especially in male mice, likely related to liver tumors. The mortalities limit the usefulness of the noncancer renal data.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical analysis was not performed for noncancer endpoints, but incidence data for independent statistical analysis are available in the individual animal pathology tables.
	Metric 24:	Reporting of Data	Low	× 2	6	incidence data are available in the individual animal pathology tables; however, severity data were not reported on either group or individual basis.

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Study Citation:	NCI (1976). Carcinogenesis bioassay of trichloroethylene
Data Type:	2 yr gavage study in mice
HERO ID:	75178

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		~~Medium~~ ⟶ Low§		~~1.7~~	
Extracted		No			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Data on noncancer renal effects are limited by 12 week untreated period prior to sacrifice and early mortalities in male mice."

Table 57: **Animal toxicity evaluation results of Sanders et al 1982 for a chronic drinking water study in mice on hematological and immune outcomes**

Study Citation:	Sanders, VM; Tucker, AN; White, KL, Jr; Kauffmann, BM; Hallett, P; Carchman, RA; Borzelleca, JF; Munson, AE (1982). Humoral and cell-mediated immune status in mice exposed to trichloroethylene in the drinking water Toxicology and Applied Pharmacology, 62(3,3), 358-368
Data Type:	chronic dw study-TCE immunotoxicity
HERO ID:	75246

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitely.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer and lot number of the test chemical were reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Authors report using naïve animal controls and vehicle controls concurrently.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed for study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation and storage were well-described; <20% loss during 3 or 4 days and up to 45% loss over 4-day period. The solutions were changed twice weekly.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Authors report details of exposure administration; exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	Not Rated	NA	NA	Doses were reported in another publication (Tucker et al., 1981)
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and/or outcome(s) of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Study authors selected 4 dw concentrations plus control, concentrations based on a range finding study.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The route and method of exposure were reported.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain, sex and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported and appropriate.

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Study Citation:	Sanders, VM; Tucker, AN; White, KL, Jr; Kauffmann, BM; Hallett, P; Carchman, RA; Borzelleca, JF; Munson, AE (1982). Humoral and cell-mediated immune status in mice exposed to trichloroethylene in the drinking water Toxicology and Applied Pharmacology, 62(3,3), 358-368
Data Type:	chronic dw study-TCE immunotoxicity
HERO ID:	75246

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported, appropriate for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Several measures of cell-mediated and humoral immunity were reported.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcome(s) of interest were reported and the study used adequate sampling for the outcome(s) of interest.
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported; however, lack of blinding is not expected to have a substantial impact on results.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative controls were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food/water intake were not reported. These are likely reported in Tucker et al. (1981).
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for dataset(s).
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.
Overall Quality Determination[‡]			High		1.5	
Extracted			No			

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Study Citation:	Sanders, VM; Tucker, AN; White, KL, Jr; Kauffmann, BM; Hallett, P; Carchman, RA; Borzelleca, JF; Munson, AE (1982). Humoral and cell-mediated immune status in mice exposed to trichloroethylene in the drinking water Toxicology and Applied Pharmacology, 62(3,3), 358-368
Data Type:	chronic dw study-TCE immunotoxicity
HERO ID:	75246

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 58: **Animal toxicity evaluation results of Fukuda et al 1983 for a 2-yr inhalation study in rats and mice on cancer, mortality, and body weight outcomes**

Study Citation:	Fukuda, K; Takemoto, K; Tsuruta, H (1983). Inhalation carcinogenicity of trichloroethylene in mice and rats Industrial Health, 21(4), 243-254
Data Type:	2-year inhalation cancer bioassay in rats and mice
HERO ID:	75288

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance is identified by chemical name.
	Metric 2:	Test Substance Source	High	× 1	1	Source of test substance is reported (Kokusan Chemical Co.).
	Metric 3:	Test Substance Purity	High	× 1	1	99.8% TCE in vapor phase; impurities identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Use of a negative control group is reported, but details were not provided. This is not likely to have a substantial impact on interpretation of results.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control group is not needed for this type of study.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups. It is unknown whether this had a substantial impact on results.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Equipment and method used to generate test substance vapor is reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	There is no indication of differences in exposure administration among groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target concentrations (0, 50, 150, 450 ppm) were reported. Concentrations in vapor were measured by GC/FID (air sampled at half-hour intervals). "The mean exposure levels were within 2% of the target values with smaller standard deviations."
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Inhalation exposure 7 hours/day, 5 days/week for 104 weeks followed by a 3-week observation period which are appropriate for this study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups (3) and spacing (50, 150, 450 ppm) were adequate for the purpose of the study. Concentrations were chosen that "could be seen in the actual work place".
	Metric 12:	Exposure Route and Method	High	× 1	1	A dynamic, whole-body chamber was used for vapor exposure.
Domain 4: Test Organism						

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Study Citation:	Fukuda, K; Takemoto, K; Tsuruta, H (1983). Inhalation carcinogenicity of trichloroethylene in mice and rats Industrial Health, 21(4), 243-254
Data Type:	2-year inhalation cancer bioassay in rats and mice
HERO ID:	75288

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Initial body weights of rats and mice were not reported. Age was 7 weeks at study initiation. Strains of rats and mice were appropriate.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported. Information on temperature, humidity, and light-dark cycle of housing was not provided. (Temperature and humidity of inhalation chambers were reported and adequate.)
	Metric 15:	Number per Group	High	× 1	1	Number of animals (49-50 female mice/group, 49-51 female rats/group) was reported and appropriate. (Only females were used.)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Histological examination of tumors.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was carried out consistently among groups (all survivors necropsied at 107 weeks; animals that died before that were also examined, except "a few that were killed accidentally, severely autolyzed or cannibalized, and died before the first appearance of tumors among the groups").
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not required for initial histopathological review.
	Metric 20:	Negative Control Response	High	× 1	1	Tumor incidence in negative control group appears to be acceptable.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Respiratory rate was not reported, but this is not likely to have a significant impact on interpretation of results. (TCE is only a mild respiratory irritant.)
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	This metric is scored as Medium for rats and High for mice. Significantly increased mortality of control rats was observed at 85 weeks and after 100 weeks. An explanation was not provided by the study author. However, the incidence of attrition is unlikely to have a substantial impact on interpretation of results (no significant increases in tumor incidence were observed in TCE-exposed rats). In mice, no significant differences in mortality were observed among groups.
Domain 7: Data Presentation and Analysis						

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Study Citation:	Fukuda, K; Takemoto, K; Tsuruta, H (1983). Inhalation carcinogenicity of trichloroethylene in mice and rats Industrial Health, 21(4), 243-254
Data Type:	2-year inhalation cancer bioassay in rats and mice
HERO ID:	75288

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 23: Statistical Methods	Medium	× 1	2	Based on footnote in Table 2, Fisher's exact test was used for tumor incidence. A detailed description of statistical analysis was not provided.
	Metric 24: Reporting of Data	High	× 2	2	Details on tumor incidence, location, and tumor type were provided.
Overall Quality Determination‡		High		1.4	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 59: **Animal toxicity evaluation results of NTP 1990 for a 2-yr oral gavage study in rats on cancer, mortality, nutrition and metabolic/adult exposure body weight, hepatic, renal, respiratory, reproductive, hematological and immune, endocrine, neurological/behavior, cardiovascular, skin and connective tissue, thyroid, and gastrointestinal outcomes**

Study Citation:	NTP (1990). Carcinogenesis studies of trichloroethylene (without epichlorohydrin) (CAS No. 79-01-6) in F344/N rats and B6C3F1 mice (gavage studies) Technical Report Series, 243
Data Type:	2-year cancer bioassay, gavage, rats
HERO ID:	87574

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively (by name and CASRN).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported, including manufacturer and batch/lot number.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity (reported as greater than 99.9%) and composition were reported and were such that any observed effects were highly likely to be due to the nominal test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using appropriate control groups (vehicle control and untreated control groups were used in 2-year rat study).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable - Positive control is not indicated for the study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The study authors reported that animals were randomly allocated into study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation and storage conditions were reported and appropriate for the test substance. The prepared substance (in corn oil stock solution) was stored for up to one week at 2-5 deg C, storage conditions which were reported to be stable (e.g., Appendix J).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups in a scientifically sound manner.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The administered doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and outcomes of interest.

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Study Citation: NTP (1990). Carcinogenesis studies of trichloroethylene (without epichlorohydrin) (CAS No. 79-01-6) in F344/N rats and B6C3F1 mice (gavage studies) Technical Report Series, 243
Data Type: 2-year cancer bioassay, gavage, rats
HERO ID: 87574

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose spacing were justified by the study authors and considered adequate to address the purpose of the study.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Most test animal characteristics, including species, strain, sex, age, and starting body weight were reported; however, health status at the beginning of the study was not reported. The animals were obtained from a commercial source. The test species and strain were an appropriate animal model.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions, including temperature, humidity, and light-dark cycle, were reported and were adequate and the same for the control and exposed populations.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group (50/sex/dose) was reported and appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcomes of interest and was sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups using the same protocol in all study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for outcomes of interest were reported and the study used adequate sampling for outcomes.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	The histopathology evaluation was performed unblinded initially as per published reports, hence not applicable. However, study reports that the diagnoses were completed, the pathology data was verified by an independent quality assurance laboratory where an experienced rodent pathologist evaluated data from 10% of the animals. "The final diagnosis represents a consensus of contractor pathologists and the NTP Pathology Working Group."
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group was adequate.

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Study Citation:	NTP (1990). Carcinogenesis studies of trichloroethylene (without epichlorohydrin) (CAS No. 79-01-6) in F344/N rats and B6C3F1 mice (gavage studies) Technical Report Series, 243
Data Type:	2-year cancer bioassay, gavage, rats
HERO ID:	87574

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	Medium	× 2	4	There were no reported differences among the study groups in initial body weight. While food/water intakes and respiratory rate were not reported, the lack of reporting is not likely to have a significant impact on results.
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	× 1	2	The authors reported that 20% of male animals in the high-dose group were killed accidentally by gavage error.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	The statistical methods were clearly described and appropriate for the datasets.
	Metric 24: Reporting of Data	Medium	× 2	4	Data for exposure-related findings were reported for most, but not all, outcomes by exposure group and sex. Histopathology incidences for exposure-related findings were presented only in the text and severity scores were not discussed.
Overall Quality Determination‡		High		1.2	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 60: **Animal toxicity evaluation results of NTP et al 1990 for a oral gavage study in mice on cancer, mortality, nutrition and metabolic/adult exposure body weight, hepatic, renal, respiratory, reproductive, hematological and immune, endocrine, neurological/behavior, cardiovascular, skin and connective tissue, thyroid, and gastrointestinal outcomes**

Study Citation:	NTP (1990). Carcinogenesis studies of trichloroethylene (without epichlorohydrin) (CAS No. 79-01-6) in F344/N rats and B6C3F1 mice (gavage studies) Technical Report Series, 243
Data Type:	2-year cancer bioassay, gavage, mice
HERO ID:	87574

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively (by name and CASRN).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported, including manufacturer and batch/lot number.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity (reported as greater than 99.9%) and composition were reported and acceptable.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate vehicle control group (received corn oil only).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable - Positive control is not indicated for the study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The study authors reported that animals were randomly allocated into study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation and storage conditions were reported and appropriate for the test substance. The prepared substance (in corn oil stock solution) was stored for up to one week at 2-5 deg C, storage conditions which were reported to be stable (e.g., Appendix J).
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Details of exposure administration were reported and methods were consistent among the groups. However, the gavage volumes exceeded those typically used in similar studies (e.g., 1 mL/100 g body weight). The dose volume in mice, which had a mean initial weight of 27 g (males) and 21-22 g (females), was 0.5 mL, and this exceeded dosing volumes in similar studies for all groups of males and females throughout the study. Due to these deficiencies, I downgraded the score of this metric to low.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The administered doses were reported without ambiguity.

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Study Citation: NTP (1990). Carcinogenesis studies of trichloroethylene (without epichlorohydrin) (CAS No. 79-01-6) in F344/N rats and B6C3F1 mice (gavage studies) Technical Report Series, 243
Data Type: 2-year cancer bioassay, gavage, mice
HERO ID: 87574

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Unacceptable	× 1	4	This part of the study in mice has only one dose group in addition to the control group. As per PECO any study that has "Only 1 quantitative dose or concentration level in addition to the control" is not acceptable.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Most test animal characteristics, including species, strain, sex, age, and starting body weight were reported; however, health status at the beginning of the study was not. The animals were obtained from a commercial source. The test species and strain were an appropriate animal model.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions, including temperature, humidity, and light-dark cycle, were reported and were adequate and the same for the control and exposed populations.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group (50/sex/dose) was reported and appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcomes of interest and was sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups using the same protocol in all study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for outcomes of interest were reported and the study used adequate sampling for outcomes.
	Metric 19:	Blinding of Assessors	High	× 1	1	Not applicable - No subjective outcome evaluations were described. Histopathology changes were not described as re-evaluation, so I considered this metric not applicable.

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Study Citation:	NTP (1990). Carcinogenesis studies of trichloroethylene (without epichlorohydrin) (CAS No. 79-01-6) in F344/N rats and B6C3F1 mice (gavage studies) Technical Report Series, 243
Data Type:	2-year cancer bioassay, gavage, mice
HERO ID:	87574

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group was adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among the study groups in initial body weight. While food/water intakes and respiratory rate were not reported, the lack of reporting is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The statistical methods were clearly described and appropriate for the datasets.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data for exposure-related findings were reported for most, but not all, outcomes by exposure group and sex. Histopathology incidences for exposure-related findings were presented only in the text and severity scores were not discussed.
Overall Quality Determination‡			~~Unacceptable~~** ⟶ Unacceptable§		~~1.3~~	
Extracted			No			

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Given that dosing volumes exceeded those typically used in similar studies and only one quantitative dose was administered, this study is not acceptable."

Table 61: **Animal toxicity evaluation results of Maltoni et al 1986 for a 104-wk inhalation carcinogenicity study in rats on cancer outcomes**

Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	104-week inhalation carcinogenicity study in rats
HERO ID:	196223

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported, and it was stated that it was analyzed (batch number and other details not provided).
	Metric 3:	Test Substance Purity	Medium	× 1	2	The test substance was reportedly "highly purified;" percent purity was not reported. minor uncertainties regarding purity are not expected to impact the results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors reported using concurrent negative controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not required by study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The study indicated that animals were randomly distributed into groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The methods and equipment used to generate the test substance were reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were provided; it appears that animals were exposed consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Actual concentrations were not reported, but there is high confidence that animals were exposed at approximately the reported target concentrations. The study states that concentrations were checked by continuous gas-chromatograhic monitoring. Records of concentrations are presumably available (conserved in archives). CK: During the course of the treatment, the concentrations and distribution of TCE were checked by continuous gas-chromatographic monitoring.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure (7 hours/day, 5 days/week, for 104 weeks) was appropriate for the outcome of interest.

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Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	104-week inhalation carcinogenicity study in rats
HERO ID:	196223

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and concentrations were justified by the study authors and considered adequate to address the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic air chambers were used, providing 12-15 air changes/hour.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain, sex, and starting body weights (presented graphically in the Appendix) were provided. The test animal species, strain and sex were adequately described and appropriate for evaluation of specific outcome
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were provided and were adequate and the same for exposed groups and controls.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported, and consistent with studies of the same type (i.e., >50/sex/group for carcinogenicity studies). Two experiments were conducted in the same way and at the same time. Although one of the studies only used 40/sex/group (less than 50/sex/group recommended for this study type), the other study used 90/sex/group; results were analyzed separately and together.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcome of interest (cancer); the timing of the assessment was at spontaneous death so that the potential for neoplastic effects could be evaluated.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There were differences in the timing of the assessment (animals were allowed to live until spontaneous death), but the outcome assessment protocol was consistent across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals were subjected to histopathological examinations (> 35 tissues); carcinogenicity was assessed in an adequate number of animals/group.
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding is not required for initial histopathology review; however, the study indicated that slides were screened by a junior pathologist and reviewed by the same senior pathologist throughout the study.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control groups were adequate.

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Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	104-week inhalation carcinogenicity study in rats
HERO ID:	196223

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no reported differences among study groups with respect to initial body weights. Lack of data on respiratory rates in not likely to impact the results. A major issue associated with this laboratory (Ramazzini Institute) is that animals are not sacrificed together at the end of dosing but were kept under observation until spontaneous death. This could result in infection or autophagy, which may result in false positive lesions. However, because there was random allocation to study groups, the observed dose-response is expected to be independent of this confounder. There was an absence of kidney lesions observed in the control or lowest dose, indicating that background false positives were not an issue.
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data for animal attrition were provided (graphically) in the Appendix; there are not differences that would influence the outcome assessment. Downgraded because potential infection may have resulted due to not immediately sacrificing the animals, as was identified by the NTP Pathology Working Group in a 2011 review of pathology results from other Ramazzini Institute studies. The NTP review primarily found significant effects on inflammatory cancers (blood, respiratory tract) as opposed to solid tumors.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Statistical methods were described and appropriate for the datasets of interest.
	Metric 24: Reporting of Data	High	× 2	2	Data for exposure-related findings were reported for outcomes by exposure and sex.
Overall Quality Determination[‡]		~~High~~ ⟶ Medium[§]		~~1.3~~	
Extracted		Yes			

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Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	104-week inhalation carcinogenicity study in rats
HERO ID:	196223

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Downgraded due to laboratory protocols allowing continued observation until spontaneous death, which may result in infection or autophagy and complicated the interpretation of results. For the kidney effects identified in this study, there are unlikely to be significant artifacts that would detract from the results, so the study is still of Medium quality overall."

Table 62: **Animal toxicity evaluation results of Maltoni et al 1986 for a 78-wk inhalation carcinogenicity study in mice on cancer outcomes**

Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	78 week inhalation carcinogenicity study in mice
HERO ID:	196223

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported, and it was stated that it was analyzed (batch number and other details not provided).
	Metric 3:	Test Substance Purity	Medium	× 1	2	The test substance was reportedly "highly purified;" percent purity was not reported. minor uncertainties regarding purity are not expected to impact the results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors reported using concurrent negative controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not required by study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The study indicated that animals were randomly distributed into groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The methods and equipment used to generate the test substance were reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were provided; it appears that animals were exposed consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Actual concentrations were not reported, but there is high confidence that animals were exposed at approximately the reported target concentrations. The study states that concentrations were checked by continuous gas-chromatograhic monitoring. Records of concentrations are presumably available (conserved in archives).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure (7 hours/day, 5 days/week, for 78 weeks) was appropriate for the outcome of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and concentrations were justified by the study authors and considered adequate to address the outcome of interest.

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Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	78 week inhalation carcinogenicity study in mice
HERO ID:	196223

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic air chambers were used, providing 12-15 air changes/hour.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain, sex, and starting body weights (presented graphically in the Appendix) were provided. The study indicated that Swiss mice were "of the breed routinely employed in our Laboratories" (no further information provided). B6C3F1 mice were purchased from the NCI source. These species/strains are usually used in carcinogenicity assays. CK: changed from medium to high as the test animal species, strain and sex were adequately described and appropriate for evaluation of specific outcome
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were provided and were adequate and the same for exposed groups and controls.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported, and consistent with studies of the same type (i.e., >50/sex/group for carcinogenicity studies).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcome of interest (cancer); the timing of the assessment was at spontaneous death so that the potential for neoplastic effects could be evaluated.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There were differences in the timing of the assessment (animals were allowed to live until spontaneous death), but the outcome assessment protocol was consistent across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals were subjected to histopathological examinations (> 35 tissues); carcinogenicity was assessed in an adequate number of animals/group.
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding is not required for initial histopathology review; however, the study indicated that slides were screened by a junior pathologist and reviewed by the same senior pathologist throughout the study.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control groups were adequate.
Domain 6: Confounding / Variable Control						

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Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	78 week inhalation carcinogenicity study in mice
HERO ID:	196223

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no reported differences among study groups with respect to initial body weights. Lack of data on respiratory rates in not likely to impact the results. A major issue associated with this laboratory (Ramazzini Institute) is that animals are not sacrificed together at the end of dosing but were kept under observation until spontaneous death. This could result in infection or autophagy, which may result in false positive lesions. However, because there was random allocation to study groups, the observed dose-response is expected to be independent of this confounder. There was an absence of kidney lesions observed in the control or lowest dose, indicating that background false positives were not an issue.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	High, early mortality was reported in male B6C3F1 mice; reportedly because these mice were older at arrival and prone to aggressiveness and fighting. Therefore, an additional group of male B6C3F1 mice (90 males/group) were exposed to TCE under the same experimental conditions. Data for animal attrition were provided (graphically) in the Appendix; there are not differences that would influence the outcome assessment. Downgraded because potential infection may have resulted due to not immediately sacrificing the animals, as was identified by the NTP Pathology Working Group in a 2011 review of pathology results from other Ramazzini Institute studies. The NTP review primarily found significant effects on inflammatory cancers (blood, respiratory tract) as opposed to solid tumors.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were described and appropriate for the datasets of interest.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were reported for outcomes by exposure and sex.
Overall Quality Determination‡			~~High~~ ⟶ Medium§		~~1.3~~	
Extracted			Yes			

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Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	78 week inhalation carcinogenicity study in mice
HERO ID:	196223

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Downgraded due to laboratory protocols allowing continued observation until spontaneous death, which may result in infection or autophagy and complicated the interpretation of results. For the kidney effects identified in this study, there are unlikely to be significant artifacts that would detract from the results, so the study is still of Medium quality overall."

Table 63: **Animal toxicity evaluation results of Maltoni et al 1986 for a 52-wk oral carcinogenicity study in rats on cancer outcomes**

Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	52 week oral carcinogenicity study in rats
HERO ID:	196223

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported, and it was stated that it was analyzed (batch number and other details not provided).
	Metric 3:	Test Substance Purity	Medium	× 1	2	The test substance was reportedly "highly purified;" percent purity was not reported. minor uncertainties regarding purity are not expected to impact the results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors reported using concurrent negative controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not required by study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The study indicated that animals were randomly distributed into groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	All details regarding test preparation and storage conditions were not reported, but these omissions are not likely to impact study results.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Gavage volume was not reported. The study authors stated that exposures were administered consistently across study groups (early morning on Monday through Friday).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Administered doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure (4-5 times/week for 52 weeks) was appropriate for the outcome of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	An adequate number of dose groups was used; however, the study authors did not justify the doses selected (unclear if the highest dose was high enough).
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were suitable.
Domain 4: Test Organism						

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Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	52 week oral carcinogenicity study in rats
HERO ID:	196223

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain, sex, and starting body weights (presented graphically in the Appendix) were provided. The study indicated that Sprague-Dawley rats were "of the breed routinely employed in our Laboratories" (no further information provided). These species/strains are usually used in carcinogenicity assays.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were provided and were adequate and the same for exposed groups and controls.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per study group was lower than the typical number used in studies of this type (30/sex/group compared to 50/sex/group typically used for rodent bioassays), but sufficient for statistical analyses and this is unlikely to impact the study results.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcome of interest (cancer); the timing of the assessment was at spontaneous death so that the potential for neoplastic effects could be evaluated.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There were differences in the timing of the assessment (animals were allowed to live until spontaneous death), but the outcome assessment protocol was consistent across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals were subjected to histopathological examinations (> 35 tissues); carcinogenicity was assessed in an adequate number of animals/group.
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding is not required for initial histopathology review; however, the study indicated that slides were screened by a junior pathologist and reviewed by the same senior pathologist throughout the study.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control groups were adequate.
Domain 6: Confounding / Variable Control						

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Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	52 week oral carcinogenicity study in rats
HERO ID:	196223

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no reported differences among study groups with respect to initial body weights. Lack of reporting on food/water intake is not likely to significantly impact results. A major issue associated with this laboratory (Ramazzini Institute) is that animals are not sacrificed together at the end of dosing but were kept under observation until spontaneous death. This could result in infection or autophagy, which may result in false positive lesions. However, because there was random allocation to study groups, the observed dose-response is expected to be independent of this confounder. There was an absence of kidney lesions observed in the control or lowest dose, indicating that background false positives were not an issue.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	High mortality was reported in female rats (no explanation was provided). Additionally, potential infection may have resulted due to not immediately sacrificing the animals, as was identified by the NTP Pathology Working Group in a 2011 review of pathology results from other Ramazzini Institute studies. The NTP review primarily found significant effects on inflammatory cancers (blood, respiratory tract) as opposed to solid tumors.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were described and appropriate for the datasets of interest.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were reported for outcomes by exposure and sex.
Overall Quality Determination[‡]			~~High~~ ⟶ Medium[§]		~~1.4~~	
Extracted			Yes			

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Study Citation:	Maltoni, C; Lefemine, G; Cotti, G (1986). Experimental research on trichloroethylene carcinogenesis Archives of Research on Industrial Carcinogenesis, 5
Data Type:	52 week oral carcinogenicity study in rats
HERO ID:	196223

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study
§ Evaluator's explanation for rating change: "Downgraded due to laboratory protocols allowing continued observation until spontaneous death, which may result in infection or autophagy and complicated the interpretation of results. For the kidney effects identified in this study, there are unlikely to be significant artifacts that would detract from the results, so the study is still of Medium quality overall."

Table 64: **Animal toxicity evaluation results of Keil et al 2009 for a chronic drinking water study in mice on hematological and immune, renal, hepatic, and body weight outcomes**

Study Citation:	Keil, DE; Peden-Adams, MM; Wallace, S; Ruiz, P; Gilkeson, GS (2009). Assessment of trichloroethylene (TCE) exposure in murine strains genetically-prone and non-prone to develop autoimmune disease Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 44(5), 443-453
Data Type:	Immunotoxicity screen - B6C3F1 mice
HERO ID:	486801

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Trichlorethylene
	Metric 2:	Test Substance Source	High	× 1	1	Sigma. Batch no. not reported. No analytical report. Batch not required because TCE does not vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Drinking water with vehicle
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not required for study type
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	TCE mixed with drinking water using 1% emulphor vehicle. Drinking water solutions replaced every 3 days to account for evaporation, and TCE levels were confirmed. Storage conditions not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	All exposed via drinking water. Drinking water solutions replaced every 3 days to account for evaporation, and TCE levels were confirmed
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Exposures reported in PPB 0, 1400, or 14000. Drinking water intake was not reported, and results of drinking water intake not reported,. Only terminal BW reported, so strain/species defaults for BW and water intake would be needed to calculated doses in mg/kg-d. Doses were derived in EPA IRIS Assessment (2011).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	From week 9-39 ; drinking water ad libitum
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 exposure, 1 control. The TCE concentrations chosen were based on noncancer benchmark dose (BMD) calculations. Similar to levels found at several National Priority List (NPL) sites. The study could have benefitted from adding a lower dose, since effects were seen at all doses and were not consistently dose-responsive.

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Study Citation:	Keil, DE; Peden-Adams, MM; Wallace, S; Ruiz, P; Gilkeson, GS (2009). Assessment of trichloroethylene (TCE) exposure in murine strains genetically-prone and non-prone to develop autoimmune disease Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 44(5), 443-453
Data Type:	Immunotoxicity screen - B6C3F1 mice
HERO ID:	486801

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	Drinking water. Frequent changing of water with exposure level analysis to avoid decreased dosing due to vaporization.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Female B6C3F1 mice (Jackson Lab). 9 wks old at study initiation. Beginning BW not reported. (Qualitative statement that initial BW did not differ between groups). Note: NZBWF1 mice were also tested in this study; this mouse strain would be considered unacceptable by this metric because it spontaneously develops autoimmune disease. This form is for the B5C3F1 mice only.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Housing, temp, humidity, and dark/light cycle reported. Water and feed ad libitum. Same between groups.
	Metric 15:	Number per Group	Medium	× 1	2	10 females/group. 10 is less than the typical recommended number of animals per group for subchronic (20 total, 10 females and 10 males according to OECD) or chronic (40 total, 20 and 20 according to OPPTS guidelines). Downgraded to medium.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Renal: organ weight and histo [HIGH] Hepatic: organ weight [MEDIUM] Immune: spleen and thymus weight and cellularity, natural killer (NK) cell activity, total IgG levels, autoantibody production, T-cell activation, and lymphocytic proliferative responses [HIGH] So high for all endpoints except liver, which was not the main focus of this study. Selected "high" because the focus of the study (immunotox) had multiple outcomes assessed. Note: If hepatic was evaluated on its own, the rating for this metric would be medium, but the overall metric for the entire study would still be high, so I kept all endpoints together.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent across groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	10/group. No information provided about technical replicates. Downgraded to medium.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not needed for outcomes assessed.

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Study Citation:	Keil, DE; Peden-Adams, MM; Wallace, S; Ruiz, P; Gilkeson, GS (2009). Assessment of trichloroethylene (TCE) exposure in murine strains genetically-prone and non-prone to develop autoimmune disease Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 44(5), 443-453
Data Type:	Immunotoxicity screen - B6C3F1 mice
HERO ID:	486801

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Negative Control Response	High	× 1	1	Control data reported; no deviation from expected reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Qualitative statement regarding no difference in initial BW; no changes in terminal BW. Water and food consumption not reported (but due to lack of changes in BW, it is not likely that there was a >20% difference). Therefore, this is not likely to have a substantial impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Data were tested for normality (Shapiro-WilksW-test) and homogeneity (Bartlett's test for unequal variances) and, if needed, appropriate transformations were performed. A one-wayANOVAwas used to determine differences among doses for each endpoint. When significant differences were detected by the F-test ($p < 0.05$), Dunnett's t-test was used to compare treatment groups to the control group
	Metric 24:	Reporting of Data	Medium	× 2	4	All significantly altered findings reported quantitatively in tables or figures except thymic cellularity (qualitative reporting of significant decrease)
Overall Quality Determination‡			High		1.7	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 65: **Animal toxicity evaluation results of Maltoni et al 1986 for a 2-yr carcinogenicity bioassay - oral - rats study on cancer outcomes**

Study Citation:	Maltoni, C; Cotti, G (1986). Results of long-term carcinogenicity bioassays of tetrachloroethylene on Sprague-Dawley rats administered by ingestion Acta Oncologica (Italy), 7(1), 11-26
Data Type:	2-year carcinogenicity bioassay - oral - rats
HERO ID:	630745

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as TTCE (tetrachloroethylene) Note: This study has been listed under TCE, but the chemical compound test is Tetrachloroethyele (Perc)
	Metric 2:	Test Substance Source	Low	× 1	3	Omitted details on the source of the test substance
	Metric 3:	Test Substance Purity	Medium	× 1	2	several impurities have been reported in the test chemical; carbon tetrachloride (53 ppm), 1,1,2-trichloroethane (11 ppm), and asymmetrical tetrachloroethane (20 ppm). They may not have substantial impact on the results
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Extra-virgin olive oil was used as a vehicle control
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for this study type
	Metric 6:	Randomized Allocation	Medium	× 1	2	random allocation was noted as "divided into groups by litter distribution".
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Some preparation information was reported. No storage information was provided
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	The animals were exposed once daily, 4-5 days weekly, for 104 weeks
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	The dose tested was reported (500 mg/kg/bw), however, only one dose was tested
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	daily (4-5 days per week) for 104 weeks
	Metric 11:	Number of Exposure Groups and Dose Spacing	Unacceptable	× 1	4	Only one dose tested; the single dose was not justified by the study authors. CK: Also, according to PECO, at least two dose groups are needed
	Metric 12:	Exposure Route and Method	High	× 1	1	gavage
Domain 4: Test Organism						

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Study Citation:	Maltoni, C; Cotti, G (1986). Results of long-term carcinogenicity bioassays of tetrachloroethylene on Sprague-Dawley rats administered by ingestion Acta Oncologica (Italy), 7(1), 11-26
Data Type:	2-year carcinogenicity bioassay - oral - rats
HERO ID:	630745

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The source of test animals was unclear; animals were noted to be the same breed used for bioassays in the experimental laboratories of the author's institute; unclear the impact on results. strain, sex and age were reported. Animals were examined throughout the study.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate. Only temperature was reported; humidity and light-dark cycle were not reported; unclear the impact on results.
	Metric 15:	Number per Group	High	× 1	1	50/sex for control group; 40/sex for treatment group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	assessment made for each treated and control animal
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not rated/applicable; initial histopathology evaluation
	Metric 20:	Negative Control Response	Medium	× 1	2	There was a slightly higher number of tumors in control rats than in treated groups.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No notable confounding variables
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical analysis was not described clearly
	Metric 24:	Reporting of Data	High	× 2	2	average body weight, tumors at various sites were reported
Overall Quality Determination‡			Unacceptable⋆⋆		1.6	
Extracted			Yes			

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Study Citation:	Maltoni, C; Cotti, G (1986). Results of long-term carcinogenicity bioassays of tetrachloroethylene on Sprague-Dawley rats administered by ingestion Acta Oncologica (Italy), 7(1), 11-26
Data Type:	2-year carcinogenicity bioassay - oral - rats
HERO ID:	630745

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

6 Genetic toxicity studies

Table 66: **Animal toxicity evaluation results for Miller and Adler 1992 for aneuploidy and mitotic delay in mouse spermatocytes**

Study Citation:	B. M. Miller, I. D. Adler (1992). Aneuploidy induction in mouse spermatocytes Mutagenesis, 7(1,1), 69-76					
Data Type:	Aneuploidy and mitotic delay in mouse spermatocytes for chloral hydrate					
HERO ID:	874					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate.
	Metric 2:	Test Substance Source	Medium	× 1	2	The donor source of the test substance was identified. It was not reported whether the test substance was synthesized commercially or by the donor laboratory.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Concurrent negative controls were used, but it was unclear whether negative control animals were treated with vehicle or left untreated.
	Metric 5:	Positive Controls	High	× 1	1	The study included two positive control substances, which responded appropriately. One positive control substance was colchicine. It was unclear which test substance served as the other positive control.
	Metric 6:	Randomized Allocation	High	× 1	1	Randomized allocation of animals to experimental groups was reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance preparation was reported and appropriate. The storage of the test substance was not reported, but this is appropriate based on the study design (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for this endpoint (single dose; sample collection at 6, 14, and 22 hr post-injection).
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only one dose of chloral hydrate was tested (200 mg/kg).
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route and duration were appropriate for the test substance.
Domain 4: Test Organism						

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Study Citation: B. M. Miller, I. D. Adler (1992). Aneuploidy induction in mouse spermatocytes Mutagenesis, 7(1,1), 69-76
Data Type: Aneuploidy and mitotic delay in mouse spermatocytes for chloral hydrate
HERO ID: 874

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The test animal species, strain, sex, age, and starting body weight range were reported. The test animal commercial source was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was appropriate for these endpoints (n = 6).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest (100 metaphase II cells from each animal for assessment of aneuploidy, and 1000 nuclei at midpachytene for assessment of mitotic or meiotic delay).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric was not applicable to the study design.
	Metric 20:	Negative Control Response	High	× 1	1	Negative responses were observed in negative control groups.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food, and water intake were not reported across groups.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The data were analyzed appropriately for each endpoint.
	Metric 24:	Reporting of Data	High	× 2	2	All data were adequately reported.
Overall Quality Determination‡			Medium		1.7	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 67: **Animal toxicity evaluation results for Clay 2008 for comet assay study in rat kidney**

Study Citation:	P. Clay (2008). Assessment of the genotoxicity of trichloroethylene and its metabolite, S-(1,2-dichlorovinyl)-L-cysteine (DCVC), in the comet assay in rat kidney Mutagenesis, 23(1,1), 27-33					
Data Type:	Rat kidney Comet assay for TCE					
HERO ID:	729644					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name as trichloroethylene (TCE).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance (BDH) was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported to be 99.5%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control groups were included (air).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not required for study type. During the oral experiment with DCVC, concurrent positive controls were included. An in vitro positive control experiment was also conducted on cells from vehicle control animals by exposing them to N-methyl-N'-nitro-N-nitrosoguanidine.
	Metric 6:	Randomized Allocation	Low	× 1	3	The method of allocation of animals was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Equipment and methodology of atmosphere generation were described in detail, and chamber concentrations measured hourly during exposure.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was reported to be consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity. Nominal/target concentrations were 500, 100, and 2000 ppm; 484, 1035, and 1749 ppm were the measured analytical concentrations.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration were reported and appropriate for this endpoint (6 hr/day for 5 days).

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Study Citation:	P. Clay (2008). Assessment of the genotoxicity of trichloroethylene and its metabolite, S-(1,2-dichlorovinyl)-L-cysteine (DCVC), in the comet assay in rat kidney Mutagenesis, 23(1,1), 27-33
Data Type:	Rat kidney Comet assay for TCE
HERO ID:	729644

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups (3 plus control) and dose spacing (factors of 2) were reported and appropriate. The maximum concentration was 4X higher than the concentration inducing kidney tumors in rats exposed chronically. Transient clinical signs were seen during exposure in the 2 highest exposure groups.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The route and method of exposure were appropriate for the test substance. Whole-body inhalation chambers were used; nose-only or head only recommended.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, commercial source, and starting body weight range of the test animals were reported. The age of the test animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported, appropriate, and consistent across groups. The number of air changes per hour (15) for the animal room was reported.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and appropriate for this study design (n = 5).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (Comet assay) was described in detail and appropriate for this endpoint.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcome of interest (3 technical replicates per animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Slide analysis was automated so this metric is not applicable
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses were reported and appeared appropriate.
Domain 6: Confounding / Variable Control						

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Study Citation:	P. Clay (2008). Assessment of the genotoxicity of trichloroethylene and its metabolite, S-(1,2-dichlorovinyl)-L-cysteine (DCVC), in the comet assay in rat kidney Mutagenesis, 23(1,1), 27-33
Data Type:	Rat kidney Comet assay for TCE
HERO ID:	729644

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Starting body weight ranges were included. Respiratory rates were not reported. Considering the known anaesthetic effects of TCE and the clinical signs seen in high exposure groups, bradypnea may have occurred resulting in different respiratory rates among exposure groups, with potential to significantly affect results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The data were analyzed by Student's t-test. The raw data, including individual animal data (mean of 3 technical replicates), are provided, enabling independent analysis.
	Metric 24:	Reporting of Data	High	× 2	2	All data were reported for all exposure groups (individual animal data, mean of 3 slides/animal)
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 68: **Animal toxicity evaluation results for Clay 2008 for comet assay study in rat kidney**

Study Citation:	P. Clay (2008). Assessment of the genotoxicity of trichloroethylene and its metabolite, S-(1,2-dichlorovinyl)-L-cysteine (DCVC), in the comet assay in rat kidney Mutagenesis, 23(1,1), 27-33					
Data Type:	Rat kidney Comet assay for DCVC					
HERO ID:	729644					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name as S-(1,2-dichlorovinyl)-L-cysteine (DCVC).
	Metric 2:	Test Substance Source	Medium	× 1	2	The test substance was synthesized in-house and its identity was confirmed with a purity of >95%. It was not reported what analytical methods were utilized to determine the purity of the test substance.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported to be >95%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative vehicle control group was included.
	Metric 5:	Positive Controls	High	× 1	1	Concurrent positive control was included (N-nitrosodimethylamine gavage).
	Metric 6:	Randomized Allocation	Low	× 1	3	The method of allocation of animals was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Preparation of the test substance was briefly reported. While authors noted use of vehicle control, the vehicle was not specified. Storage of the test substance was not reported but this is not expected to impact results (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was reported to be consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (1 or 10 mg/kg bw)
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration (single gavage dose) were reported and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 doses plus control used. High dose selected to yield ~1000 fold higher than amount formed in rats exposed to TCE
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure (oral gavage) were justified by the authors and appropriate for the test substance.
Domain 4: Test Organism						

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Study Citation:	P. Clay (2008). Assessment of the genotoxicity of trichloroethylene and its metabolite, S-(1,2-dichlorovinyl)-L-cysteine (DCVC), in the comet assay in rat kidney Mutagenesis, 23(1,1), 27-33
Data Type:	Rat kidney Comet assay for DCVC
HERO ID:	729644

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, commercial source, and starting body weight range of the test animals were reported. The age of the test animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported, appropriate, and consistent across groups.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and appropriate for this study design (n = 5).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (Comet assay) was described in detail and appropriate for this endpoint.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcome of interest (3 technical replicates per animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Slide analysis was automated so this metric is not applicable
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses were reported and appeared appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Starting body weight ranges were included. Food/water consumption were not reported, but this is not likely to significantly impact results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The data were analyzed by Student's t-test. The raw data, including individual animal data (mean of 3 technical replicates), are provided, enabling independent analysis.
	Metric 24:	Reporting of Data	High	× 2	2	All data were reported for all exposure groups (individual animal data, mean of 3 slides/animal)
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	P. Clay (2008). Assessment of the genotoxicity of trichloroethylene and its metabolite, S-(1,2-dichlorovinyl)-L-cysteine (DCVC), in the comet assay in rat kidney Mutagenesis, 23(1,1), 27-33
Data Type:	Rat kidney Comet assay for DCVC
HERO ID:	729644

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 69: **Animal toxicity evaluation results for Russo and Levis 1992 for acute intraparietal study in mice on chromosomal aberations**

Study Citation:	A. Russo, A. G. Levis (1992). Further evidence for the aneuploidogenic properties of chelating agents: Induction of micronuclei in mouse male germ cells by EDTA Environmental and Molecular Mutagenesis, 19(2,2), 125-131					
Data Type:	aneuploidy, micronuclei and chromosome aberrations in mice					
HERO ID:	730035					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chloral hydrate identified by established nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer (Fluka Chemie AG) was identified. Batch/lot number was not given; however, the composition is not expected to vary.
	Metric 3:	Test Substance Purity	High	× 1	1	99% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	An untreated concurrent control group was used
	Metric 5:	Positive Controls	High	× 1	1	Adriamycin and mitomycin C were used as positive controls
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Preparation of the test substance is not well described (vehicle is not reported). Solutions were prepared immediately before injection.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Exposures were consistent across groups, except that a sham-treated control was not used
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose (83 mg/kg bw) was unambiguous.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single injection; animals were sacrificed at 24 and 48h.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single dose; justification was not provided. Dose was sufficient to induce increase in MN
	Metric 12:	Exposure Route and Method	Low	× 1	3	I.p. injection is acceptable but not recommended route of exposure
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain sex, and age were reported. Health status and starting body weight were not given.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.

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Study Citation:	A. Russo, A. G. Levis (1992). Further evidence for the aneuploidogenic properties of chelating agents: Induction of micronuclei in mouse male germ cells by EDTA Environmental and Molecular Mutagenesis, 19(2,2), 125-131
Data Type:	aneuploidy, micronuclei and chromosome aberrations in mice
HERO ID:	730035

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	At least 3/group (5 recommended) ; 2 independent experiments.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The assessment methods were partially reported with some references to other publications, and appeared to be sensitive for the outcomes of interest
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Outcomes were assessed consistently across groups; some assessment methods cited to another publication.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	1000 spermatids assessed for micronuclei; 2000 PCE/animal for bone marrow micronuclei; 100 metaphases per animal for chromosome aberrations
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applcable to the outcomes of interest. Some methods cited to other publications.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses were reported and appeared appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	No reporting of differences in initial body weight or drinking water/food consumption; however, this missing information is not likely to have a significant impact on the results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analyses were reported and appeared appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data were fully reported for all groups and endpoints (mean, SE, and n).
Overall Quality Determination‡			Medium		1.9	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 70: **Animal toxicity evaluation results for Marrazzini et al 1994 for micronucleus study**

Study Citation:	A. Marrazzini, C. Betti, F. Bernacchi, I. Barrai, R. Barale (1994). Micronucleus test and metaphase analyses in mice exposed to known and suspected spindle poisons Mutagenesis, 9(6,6), 505-515
Data Type:	in vivo MN and CA analysis in mice - CH
HERO ID:	730163

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name as Chloral Hydrate (CH).
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was reported as "distributed by the project coordinator"; a commercial source was not identified. Analytical confirmation of the test substance identity was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were tested using distilled water
	Metric 5:	Positive Controls	High	× 1	1	Positive controls were used (colchicine and vinblastin for aneuploidy induction and hydroquinone for chromosomal aberrations).
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomized in groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation was reported; freshly prepared directly prior to use
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups for both i.p. and oral exposure.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (mg/kg bw).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for this endpoint (single adminisration)
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	A stepwise protocol was used to determine the number of exposure groups. The number of exposure groups and spacing of exposure levels appear to be adequate to show results relevant to the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure routes (i.p. and oral) were appropriate for the test substance.
Domain 4: Test Organism						

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Study Citation:	A. Marrazzini, C. Betti, F. Bernacchi, I. Barrai, R. Barale (1994). Micronucleus test and metaphase analyses in mice exposed to known and suspected spindle poisons Mutagenesis, 9(6,6), 505-515
Data Type:	in vivo MN and CA analysis in mice - CH
HERO ID:	730163

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, age, and starting body weight were reported while health status was not. The test animal was from a reported commercial source. The test species and strain were an appropriate animal model for the evaluation of this endpoint. The uncertainties in reporting are unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and if differences occurred between control and exposed populations.
	Metric 15:	Number per Group	Low	× 1	3	The number of animals per study group was reported and somewhat low (2 or 3/group) for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were described and appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across dose groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Sampled numbers for MN (3,000 MnPCE scored/mouse) and CA (100 cells/animal) were lower than recommended (4000 and 200, respectively)
	Metric 19:	Blinding of Assessors	High	× 1	1	Authors report using coded slides
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the controls were reported and appeared adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Not Rated	NA	NA	Food and water consumption were not reported, but this is is not expected to influence study results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were described and appropriate for the dataset.
	Metric 24:	Reporting of Data	Low	× 2	6	CH was administered by i.p. and oral routes; however, the results appear to be reported for a single route only (i.p. injection as suggested by text).
Overall Quality Determination[‡]			High		1.6	

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Study Citation:	A. Marrazzini, C. Betti, F. Bernacchi, I. Barrai, R. Barale (1994). Micronucleus test and metaphase analyses in mice exposed to known and suspected spindle poisons Mutagenesis, 9(6,6), 505-515
Data Type:	in vivo MN and CA analysis in mice - CH
HERO ID:	730163

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 71: **Animal toxicity evaluation results for Palbykin et al 2011 for rat embryonic study on DNA methylation**

Study Citation:	Palbykin, B., Borg, J., Caldwell, P.T., Rowles, J., Papoutsis, A.J., Romagnolo, D.F., Selmin, O.I. (2011). Trichloroethylene induces methylation of the Serca2 promoter in H9c2 cells and embryonic heart Cardiovascular Toxicology, 11(3), 204-214
Data Type:	DNA methylation in rat embryo cardiac tissue after in utero exposure for TCE
HERO ID:	2128264

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name as trichloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source (Sigma-Aldrich) was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Study was comparing effects of TCE low or high folate diet. Animals received either low or high folate diet for four weeks prior to being further allocated to control and TCE exposure groups. It is not clear how the low and high folate contents compare to normal control folate intake.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable to study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The method of animal allocation to groups was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage were reported and efforts made to mitigate loss of test chemical: "TCE solutions were prepared daily in glass bottles that had been soaked in concentrated TCE solution overnight,rinsed and dried in a chemical hood before use. Each bottle was placed in a metal casing to reduce light exposure and subsequent chemical breakdown"
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	No information on water intake was provided. It is unknown whether palatability affected water intake.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Dose was reported as water concentration (10 ppb) without body weight or water intake information.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Animals were exposed from GD0 to GD10.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single exposure level was used; justified as environmentally significant.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Administered in drinking water; TCE may have volatilized from the water, but new solutions were prepared daily to minimize losses.
Domain 4: Test Organism						

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Study Citation:	Palbykin, B., Borg, J., Caldwell, P.T., Rowles, J., Papoutsis, A.J., Romagnolo, D.F., Selmin, O.I. (2011). Trichloroethylene induces methylation of the Serca2 promoter in H9c2 cells and embryonic heart Cardiovascular Toxicology, 11(3), 204-214
Data Type:	DNA methylation in rat embryo cardiac tissue after in utero exposure for TCE
HERO ID:	2128264

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species, strain, sex, and commercial source were reported; initial body weight and health status were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and if differences occurred between control and exposed populations.
	Metric 15:	Number per Group	High	× 1	1	There were 6 to 9 dams/group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Methods for assessing DNA methylation of the Serca2 promoter and S-adenosyl methionine availability were described in detail.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Consistent outcome assessment across groups is inferred from the text.
	Metric 18:	Sampling Adequacy	High	× 1	1	"Cardiac tissue was removed from each viable embryo, pooled (three groups of hearts from 2 to 3 different dams were pooled together) , and used for genomic DNA extraction". Remaining tissue used for SAM assay. Serca2 promoter methylation evaluated in 15-30 clones per exposure group.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to study type.
	Metric 20:	Negative Control Response	High	× 1	1	Control responses were reported and appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The lack of reporting of initial body weights and food/water intake is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	Statistical analysis was not performed but is not necessary for the outcomes assessed.
	Metric 24:	Reporting of Data	High	× 2	2	Data shown graphically for all experimental groups and outcomes. SAM reported with error bars.
Overall Quality Determination‡			Medium		1.9	
Extracted			Yes			

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Study Citation:	Palbykin, B., Borg, J., Caldwell, P.T., Rowles, J., Papoutsis, A.J., Romagnolo, D.F., Selmin, O.I. (2011). Trichloroethylene induces methylation of the Serca2 promoter in H9c2 cells and embryonic heart Cardiovascular Toxicology, 11(3), 204-214
Data Type:	DNA methylation in rat embryo cardiac tissue after in utero exposure for TCE
HERO ID:	2128264

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 72: **Animal toxicity evaluation results of Wilmer et al 2014 for an in vivo inhalation exposure genotoxicity (rat) study on mortality, neurological/behavior, nutrition, metabolic, and body weight outcomes**

Study Citation:	Wilmer, JW; Spencer, PJ; Ball, N; Bus, JS (2014). Assessment of the genotoxicity of trichloroethylene in the in vivo micronucleus assay by inhalation exposure Mutagenesis, 29(3), 209-214
Data Type:	In vivo genotoxicity- short term inhalation exposure - mortality, neurological, body weight)
HERO ID:	2799593

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively (name and CASRN) and synonyms.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported; however, the batch/lot number was not reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was acceptable (99.97% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative control group was used and was appropriate.
	Metric 5:	Positive Controls	High	× 1	1	Concurrent positive control groups were used and a positive response was observed.
	Metric 6:	Randomized Allocation	Medium	× 1	2	The study reported that the rats were randomized by a stratified randomization procedure using body weight and distributed into the various treatment groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation conditions were reported, but storage conditions were not. For this inhalation study, the method and equipment used to generate the test substance as a vapor were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups (e.g., similar exposure chambers).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity. Vapor concentrations were measured analytically and reported along with target concentrations. The analytical method used to measure chamber test substance was reported and appropriate.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were reported and appropriate for the study type and outcomes of interest.

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Study Citation:	Wilmer, JW; Spencer, PJ; Ball, N; Bus, JS (2014). Assessment of the genotoxicity of trichloroethylene in the in vivo micronucleus assay by inhalation exposure Mutagenesis, 29(3), 209-214
Data Type:	In vivo genotoxicity- short term inhalation exposure - mortality, neurological, body weight)
HERO ID:	2799593

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and concentration spacing were justified by the study authors and considered adequate to address the purpose of the study.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and suited to the test substance. A whole body chamber was used and acceptable for the vapor generated test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal, strain, sex, age, and body weight upon receipt were reported; however, starting body weights, after at least 7 days of acclimation, were not reported. Health status at the start of the study was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Temperature and humidity ranges were not reported; however, photocycle, 12h light/dark cycle was reported.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was reported and appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcomes of interest and was sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for outcomes of interest were reported and the study used adequate sampling for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were reported.
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the negative control group was reported and acceptable.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food/water intake were not reported. Lack of measurement of respiratory rate is considered to have a substantial impact on results because TCE is a potential respiratory irritant. The study results did report "altered respiration" at 5000 ppm as one of the clinical signs observed, but it is not mentioned whether quantitative respiratory measurements were performed.

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Study Citation:	Wilmer, JW; Spencer, PJ; Ball, N; Bus, JS (2014). Assessment of the genotoxicity of trichloroethylene in the in vivo micronucleus assay by inhalation exposure Mutagenesis, 29(3), 209-214
Data Type:	In vivo genotoxicity- short term inhalation exposure - mortality, neurological, body weight)
HERO ID:	2799593

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for the datasets.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data for exposure-related findings were presented for most, but not all, outcomes by exposure group. Incidences for clinical signs were not reported. The minor uncertainties in outcome reporting are unlikely to have a substantial impact on results.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 73: **Animal toxicity evaluation results for Wilmer et al 2014 for acute inhalation study in rats on genotoxicity outcomes**

Study Citation:	Wilmer, JW; Spencer, PJ; Ball, N; Bus, JS (2014). Assessment of the genotoxicity of trichloroethylene in the in vivo micronucleus assay by inhalation exposure Mutagenesis, 29(3), 209-214
Data Type:	In vivo genotoxicity (inhalation-genotoxicity outcome)
HERO ID:	2799593

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively (name and CASRN).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported; however, the batch/lot number was not reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was acceptable (99.97% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative control group was used and was appropriate.
	Metric 5:	Positive Controls		× 1	NA	Concurrent positive control groups were used and a positive response was observed.
	Metric 6:	Randomized Allocation	Medium	× 1	2	The study reported methods of allocation to study groups, but there were minor limitations in the allocation method (body weight was considered for allocation to groups).
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation conditions were reported, but storage conditions were not. For this inhalation study, the method and equipment used to generate the test substance as a vapor were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups (e.g., similar exposure chambers).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity. Vapor concentrations were measured analytically and reported along with target concentrations. The analytical method used to measure chamber test substance was reported and appropriate.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were reported and appropriate for the study type and outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and concentration spacing were justified by the study authors and considered adequate to address the purpose of the study.

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Study Citation:	Wilmer, JW; Spencer, PJ; Ball, N; Bus, JS (2014). Assessment of the genotoxicity of trichloroethylene in the in vivo micronucleus assay by inhalation exposure Mutagenesis, 29(3), 209-214
Data Type:	In vivo genotoxicity (inhalation-genotoxicity outcome)
HERO ID:	2799593

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and suited to the test substance. A whole body chamber was used and acceptable for the vapor generated test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal, strain, sex, age, and body weight upon receipt were reported; however, starting body weights, after at least 7 days of acclimation, were not reported. Health status at the start of the study was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were incompletely reported. Temperature and humidity ranges were not reported; however, photocycle was reported.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was reported and appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcomes of interest and was sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for outcomes of interest were reported and the study used adequate sampling for the outcomes of interest.
	Metric 19:	Blinding of Assessors	High	× 1	1	To control for bias, all slides were coded, scored, and decoded upon completion for slide scoring for genotoxicity evaluations.
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the negative control group was reported and acceptable.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food/water intake were not reported. Lack of measurement of respiratory rate is considered to have a substantial impact on results because TCE is a potential respiratory irritant. The study results did report "altered respiration" at 5000 ppm as one of the clinical signs observed, but it is not mentioned whether quantitative respiratory measurements were performed.

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Study Citation:	Wilmer, JW; Spencer, PJ; Ball, N; Bus, JS (2014). Assessment of the genotoxicity of trichloroethylene in the in vivo micronucleus assay by inhalation exposure Mutagenesis, 29(3), 209-214
Data Type:	In vivo genotoxicity (inhalation-genotoxicity outcome)
HERO ID:	2799593

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for the datasets.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.
Overall Quality Determination‡			High		0.0	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 74: **In vitro evaluation results of Mortelmans et al 1986 for bacterial reverse mutation**

Study Citation:	K. Mortelmans, S. Haworth, T. Lawlor, W. Speck, B. Tainer, E. Zeiger (1986). Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals Environmental Mutagenesis, 8(S7,S7), 1-119
Data Type:	Bacterial reverse mutation assay for TCE
HERO ID:	7315

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was reported by name: trichloroethylene and CASRN 79-01-6 in table 1 and by structure in appendix 1
	Metric 2:	Test Substance Source	High	× 1	1	Test substance was obtained from Dow Chemical co, Lot number was not reported, however, the test substance is unlikely to vary in composition
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity reported in table 1: vendor purity-99.9+%, analyzed purity , 99+%, testing lab -EGG
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent solvent controls were tested with and without metabolic activation. Solvent controls include water, DMSO and ethanol or acetone if not soluble in water or DMSO. The solvent used for the test substance was DMSO reported in appendix 2
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were tested with and without metabolic activation: without metabolic activation: sodium azide for TA1535 and TA100, 4-nitro-o-phenylenediamine for TA98, and 9-aminoacridine for TA97 and TA1537; 2-aminoanthracene was used with all strains with hamster and rat liver metabolic activation systems.
	Metric 6:	Assay Procedures	High	× 1	1	The assay procedure was well described
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Chemical preparation was reported in detail. Chemical was provided with stability and storage conditions, specific storage conditions for the test substance were not reported; however, this is appropriate for the study design (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Administration was consistent across study groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported in table 246 of appendix 2: 0, 10, 33, 100, 333, 1000 ug/plate

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Study Citation:	K. Mortelmans, S. Haworth, T. Lawlor, W. Speck, B. Tainer, E. Zeiger (1986). Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals Environmental Mutagenesis, 8(S7,S7), 1-119
Data Type:	Bacterial reverse mutation assay for TCE
HERO ID:	7315

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was appropriate for the study type and was reported for each part of the procedure: 20 minute pre-incubation and 48 h plate incubation after exposure
	Metric 12:	Exposure Route and Method	High	× 1	1	Number of doses was adequate for the study type. Dose spacing and upper limits were based on solubility and cytotoxicity
	Metric 13:	Metabolic Activation	High	× 1	1	Testing was done in the presence and absence of S9 metabolic activation. Preparation of S9 was reported.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity and source of the S. typhimrium strains TA1535, TA1537, TA98, and TA100 were reported and appropriate. These strains are routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	Three plates per dose level were utilized.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment was appropriate for the outcome of interest
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent in protocol and timing across all dose groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were reported. The number or organisms was not reported but based on a citation it is assumed to be consistent across doses.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	Data on confounding variables not related to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was not conducted, however sufficient data were provided to allow for statistical testing.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria was cited previously and briefly described and were consistent with established practice
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity testing was reported and used to determine dose range for the test substance

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Study Citation:	K. Mortelmans, S. Haworth, T. Lawlor, W. Speck, B. Tainer, E. Zeiger (1986). Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals Environmental Mutagenesis, 8(S7,S7), 1-119
Data Type:	Bacterial reverse mutation assay for TCE
HERO ID:	7315

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	Appendix 2 table 246 reports data as mean SEM for all dose groups. Table 1 includes summary +,-,eq
Overall Quality Determination‡		High		1.0	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 75: **In vitro evaluation results for Galloway et al 1987 for Chinese hamster ovary cell sister chromatid exchange study**

Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	TCE in vitro SCE
HERO ID:	7768

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified using established nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The test substances were obtained from Litton Bionetics, Inc.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substances were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Solvent controls were employed appropriately.
	Metric 5:	Positive Controls	High	× 2	2	Two positive controls were employed (triethylenemelamine or mitomycin C and cyclophosphamide); their response was appropriate (significant increase in chromosomal aberrations).
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were well described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to this study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	General information regarding test substance preparation was included (e.g., dissolving in solvent immediately before use), but storage conditions were not provided.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Information regarding exposure administration was reported and consistency of administration across groups is inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure doses were reported for each trial.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was clearly stated and appropriate for the endpoint.
	Metric 12:	Exposure Route and Method	High	× 1	1	Dose selection was described in detail and based on preliminary growth inhibition tests, followed by observations of cell monolayer confluence and mitotic activity to maximize available metaphase cells. The number of exposure groups was consistent for the test.
	Metric 13:	Metabolic Activation	High	× 1	1	Tests were run with and without metabolic activation. Preparation of S9 mix was described in detail.
Domain 4: Test Model						

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Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	TCE in vitro SCE
HERO ID:	7768

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	Test models were described in detail and appropriate for the endpoints assessed.
	Metric 15:	Number per Group	Low	× 1	3	There was only one study group for each of the three exposure concentrations tests (i.e., no replicates).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology addressed the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment protocol was consistent across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The number of cells/dose was reported and is appropriate (50 cells/dose).
	Metric 19:	Blinding of Assessors	High	× 1	1	Test substance was supplied under code; assessors did not know its identity until after scoring.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no confounding variables in test design or procedures that were reported by study authors.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	There were no confounding variables reported unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analyses were clearly described and presented in results tables.
	Metric 23:	Data Interpretation	High	× 2	2	Data were reported in such a way as to allow interpretation of test results.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints such as induction of cell death and delay in cell cycle progression were noted, and selected exposure doses were based on relation to toxicity. However, methods of measurement for specific cytotoxicity endpoints were not described.
	Metric 25:	Reporting of Data	High	× 2	2	Data were presented for percent cells with aberrations in three ways for each exposure concentration: total, simple, and complex aberrations.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

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Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	TCE in vitro SCE
HERO ID:	7768

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 76: **In vitro evaluation results for Galloway et al 1987 for Chinese hamster ovary cell chromosomal aberration study**

Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	TCE in vitro chromosomal aberration
HERO ID:	7768

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified using established nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The test substances were obtained from Litton Bionetics, Inc.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substances were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Solvent controls were employed appropriately.
	Metric 5:	Positive Controls	High	× 2	2	Two positive controls were employed (triethylenemelamine or mitomycin C and cyclophosphamide); their response was appropriate (significant increase in chromosomal aberrations).
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were well described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to this study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	General information regarding test substance preparation was included (e.g., dissolving in solvent immediately before use), but storage conditions were not provided.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Information regarding exposure administration was reported and consistency of administration across groups is inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure doses were reported for each trial.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was clearly stated and appropriate for the endpoint.
	Metric 12:	Exposure Route and Method	High	× 1	1	Dose selection was described in detail and based on preliminary growth inhibition tests, followed by observations of cell monolayer confluence and mitotic activity to maximize available metaphase cells. The number of exposure groups was consistent for the test.
	Metric 13:	Metabolic Activation	High	× 1	1	Tests were run with and without metabolic activation. Preparation of S9 mix was described in detail.
Domain 4: Test Model						

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Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	TCE in vitro chromosomal aberration
HERO ID:	7768

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	Test models were described in detail and appropriate for the endpoints assessed.
	Metric 15:	Number per Group	Low	× 1	3	There was only one study group for each of the three exposure concentrations tests (i.e., no replicates).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology addressed the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment protocol was consistent across study groups.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	The number of cells/dose (100) was reported and is slightly less than appropriate.
	Metric 19:	Blinding of Assessors	High	× 1	1	Test substance was supplied under code; assessors did not know its identity until after scoring.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no confounding variables in test design or procedures that were reported by study authors.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	There were no confounding variables reported unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analyses were clearly described and presented in results tables.
	Metric 23:	Data Interpretation	High	× 2	2	Data were reported in such a way as to allow interpretation of test results.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints such as induction of cell death and delay in cell cycle progression were noted, and selected exposure doses were based on relation to toxicity. However, methods of measurement for specific cytotoxicity endpoints were not described.
	Metric 25:	Reporting of Data	High	× 2	2	Data were presented for percent cells with aberrations in three ways for each exposure concentration: total, simple, and complex aberrations.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	TCE in vitro chromosomal aberration
HERO ID:	7768

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 77: **In vitro evaluation results of Callen et al 1980 for S. cerevisiae mutagenicity study**

Study Citation:	D. F. Callen, C. R. Wolf, R. M. Philpot (1980). Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in Saccharomyces cerevisiae Mutation Research, 77(1,1), 55-63
Data Type:	S. cerevisiae mutagenicity for TCE
HERO ID:	10054

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported. A potential impurity of the TCE stock, epichlorohydrin, "a direct-acting mutagen which does not need metabolic activation," was noted in the discussion, and was considered by the study authors to have no effect on the results (based on experimental data). It is unclear whether the TCE stock in the present study had this impurity.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design. The test substances used in the study exhibited dose-related increased frequencies of gene mutations (indicative of effective assay conditions).
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were adequately described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported; methods took into account the volatility of the test substance (i.e., the use of screw-capped centrifuge tubes). Test substance storage was not reported, but this omission is unlikely to substantially impact the study results (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate (based on observations of positive responses). Preliminary experiments were used as an aid to determine the appropriate exposure time.

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Study Citation:	D. F. Callen, C. R. Wolf, R. M. Philpot (1980). Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in Saccharomyces cerevisiae Mutation Research, 77(1,1), 55-63
Data Type:	S. cerevisiae mutagenicity for TCE
HERO ID:	10054

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	The study used two exposure groups plus controls, and substantial toxicity was observed at the highest tested dose (leaving only one analyzable concentration).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	The study used two exposure groups plus controls, and substantial toxicity was observed at the highest tested dose (leaving only one analyzable concentration).
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity, source, and relevant genetic details for the various strains of S. cerevisiae were reported and appropriate for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	At least 5 plates were used per treatment condition.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest. The methods used permitted the detection of gene revertants, gene conversion, and mitotic recombinants.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to this study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analyses are not required by study type (data for individual plates were pooled, so that independent statistical analyses are not possible). Data were presented as the number of revertants, recombinants, or convertants per 10^5 survivors (pooled data); data for numbers of revertants, recombinants, or convertants per plate (and including a measure of variation) were not reported.
	Metric 23:	Data Interpretation	High	× 2	2	The criteria for a positive result was explicitly specified (i.e., at least a doubling of colonies compared to the controls).

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Study Citation:	D. F. Callen, C. R. Wolf, R. M. Philpot (1980). Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in Saccharomyces cerevisiae Mutation Research, 77(1,1), 55-63
Data Type:	S. cerevisiae mutagenicity for TCE
HERO ID:	10054

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Cytotoxicity Data	High	× 1	1	A measure of cytotoxicity (percent survival compared to control, measured by total number of colonies counted) was determined concurrently with the mutagenicity assay results.
	Metric 25: Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡		High		1.3	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 78: **In vitro evaluation results for Bartsch et al 1979 for mutagenicity study**

Study Citation:	H. Bartsch, C. Malaveille, A. Barbin, G. Planche (1979). Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues: Evidence for oxirane formation by P450-linked microsomal mono-oxygenases Archives of Toxicology, 41(4,4), 249-277
Data Type:	Mutagenicity for TCE
HERO ID:	10689

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name (1,1,2-trichloroethylene). A structure was also provided.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified (Merck, Darmstadt, FRG). Although a lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported (99.5%). The test substance purity was high enough that any observed effects were highly likely to be due to the nominal test substance itself. It was noted that the test substance contained no detectable amounts of epichlorohydrin and 1,2-epoxybutane (</= 1 ppm).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using a concurrent negative control group (untreated, as no vehicle was used for the gaseous exposure assay), but all conditions were not equal to those of the treated groups. For plates exposed to 5% TCE vapor, EDTA was added to the soft agar layer and/or exposure occurred after 4 hours pre-incubation at 37 C (conditions not used at other exposure concentrations or in negative controls). It was unclear why EDTA was added to this treatment group.
	Metric 5:	Positive Controls	Medium	× 2	4	The study noted that "the mutability of the strains was checked with methylmethane sulphonate and N-methyl-N'-nitro-N-nitroso-guanidine". These positive controls did not appear to have been conducted concurrently. However, some test substances did show a dose-dependent response, so it is apparent that a positive response was able to be detected.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were well-described (e.g., test conditions and incubation temperatures).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						

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Study Citation:	H. Bartsch, C. Malaveille, A. Barbin, G. Planche (1979). Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues: Evidence for oxirane formation by P450-linked microsomal mono-oxygenases Archives of Toxicology, 41(4,4), 249-277
Data Type:	Mutagenicity for TCE
HERO ID:	10689

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation/storage conditions were not described in detail; however, this would not be expected to have a substantial impact on the results given that it is a short-term study.
	Metric 9:	Consistency of Exposure Administration	Low	× 1	3	Details of exposure administration were reported. Consistent application methods using an assay modified for testing gaseous/volatile chemicals was used. However, methods were not consistent across concentrations. Bacteria were pre-incubated for 4 hours and subsequently exposed to 5% TCE under one condition only; there is evidence that the difference in methods substantially impacted the study results (i.e., different results for these conditions than the others). Because this dose (5%) was also tested without the 4 hr-preincubation, this is still considered acceptable; however, it should be noted that both treatment groups with 5% TCE had EDTA added to the soft agar layer, whereas the other doses did not.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	Exposure duration for 8% and 20% was reported (16 hours exposure followed by incubation for 48 hours) and was appropriate for the testing of gases/volatile chemicals. The exposure duration for 5% TCE was 2 hr with 4 hr preincubation (considered adequate) or 2 hr without preincubation (considered inadequate for testing volatile chemicals). The overall inconsistency in exposure duration is considered inappropriate.
	Metric 12:	Exposure Route and Method	Low	× 1	3	There were deficiencies regarding the number of exposure groups and/or dose concentration spacing (one bacterial strain exposed to three concentrations; two concentrations under the same treatment conditions).

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Study Citation: H. Bartsch, C. Malaveille, A. Barbin, G. Planche (1979). Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues: Evidence for oxirane formation by P450-linked microsomal mono-oxygenases Archives of Toxicology, 41(4,4), 249-277
Data Type: Mutagenicity for TCE
HERO ID: 10689

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Low	× 1	3	The presence of a metabolic activation system was reported in the study, but not validated (mice treated with phenobarbital only rather than PB and beta-naphthoflavone). The study indicated that bacteria were exposed to the test substance in the presence of liver S9 and in the presence or absence of "cofactors" (NADP+ and glucose 6-phosphate). There was no indication that tests were carried out in the absence of metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The source of the test model (bacterial strains) was reported (i.e., provided by Professor Ames) and the model is the most commonly used for this type of assay. It was indicated that the presence of an R factor was tested (by seeding on plates containing ampicillin); mutability of the strains was also checked.
	Metric 15:	Number per Group	Medium	× 1	2	The number of replicates per group were reported and appropriate for the study type (3-4 plates per treatment group). It is not clear whether 3 or 4 plates were used for each group, but this is not expected to have impacted results.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcome of interest (number of revertants/plate).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	It was inferred from the text that the endpoint of interest was assessed consistently.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not addressed and is not considered appropriate for the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among study group parameters (e.g., test substance, bacterial strain used) that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variable unrelated to exposure were reported or identified.
Domain 7: Data Presentation and Analysis						

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Study Citation:	H. Bartsch, C. Malaveille, A. Barbin, G. Planche (1979). Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues: Evidence for oxirane formation by P450-linked microsomal mono-oxygenases Archives of Toxicology, 41(4,4), 249-277
Data Type:	Mutagenicity for TCE
HERO ID:	10689

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Low	× 1	3	No statistical analysis was conducted. The values presented in Table 4 were the average for 3-4 plates utilizing pooled tissues from five mice. No standard deviation is provided and the number of replicates for each treatment group is not clear, so independent statistical analysis is not possible. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay, so this is still considered acceptable.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were partially reported in the results. The results report dose-related and/or 2-fold increases in revertant frequency as indicative of a positive response; however, criteria were not explicitly specified (and a less than 2-fold response was indicated as positive).
	Metric 24:	Cytotoxicity Data	High	× 1	1	The absence of a background lawn of bacteria was used as an indication of gross toxicity. Toxicity was noted at the highest tested concentration of TCE.
	Metric 25:	Reporting of Data	High	× 2	2	Data were presented for outcomes by exposure group.
Overall Quality Determination‡			~~High~~ ⟶ Low§		~~1.7~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "The inconsistency of test conditions between the 3 doses, coupled with the highest dose resulting in toxicity, renders only 1 dose usable for independent analysis. Only means were reported; therefore, independent statistical analysis is not possible and other methods of assessing a positive response, such as dose-dependence, would have to be utilized, which is not possible with only 1 usable dose. A threshold of 2-fold may still be used, so this study is still considered acceptable."

Table 79: **In vitro evaluation results for Tu et al 1985 for transformation assay in mouse embryo cells**

Study Citation:	A. S. Tu, T. A. Murray, K. M. Hatch, A. Sivak, H. A. Milman (1985). In vitro transformation of BALB/c-3T3 cells by chlorinated ethanes and ethylenes Cancer Letters, 28(1,1), 85-92
Data Type:	In vitro transformation assay for TCE
HERO ID:	17978

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name (trichloroethylene).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified (purchased from Aldrich Chemical Company and provided by Dr. Mitoma of SRI International). Although a lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity of TCE was not excplicitly specified; however, it was indicated that the purity of all test chemicals was 97% to 99%. Therefore, the purity was such that observed effects were more likely than not due to the nominal test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The use of a concurrent (untreated) control group was reported.
	Metric 5:	Positive Controls	High	× 2	2	A concurrent positive control was used and the intended positive result was induced. All plates treated with 3-methylcholanthrene (MCA) had type III foci (an acceptable level of transformation was observed).
	Metric 6:	Assay Procedures	High	× 1	1	The study authors described the methods and procedures used for the test in adequate detail. The standard procedure was reported, and the ways by which the testing of volatile chemicals (TCE as a liquid) differed from the standard procedure were described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation/storage conditions were not described in detail; however, this would not be expected to have a substantial impact on the results. It was noted that liquid volatile chemicals were added directly to plates.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.

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Study Citation: A. S. Tu, T. A. Murray, K. M. Hatch, A. Sivak, H. A. Milman (1985). In vitro transformation of BALB/c-3T3 cells by chlorinated ethanes and ethylenes Cancer Letters, 28(1,1), 85-92
Data Type: In vitro transformation assay for TCE
HERO ID: 17978

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The exposure doses/concentrations or amounts of test substance were reported without ambiguity (0, 4, 20, 100, or 250 ug/mL).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (24 hours) was reported and appears to be appropriate for the study type/outcome of interest (cell transformation).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups (4 doses plus controls) were reported. The high concentration should have induced significant cytotoxicity; however, the relative surviving fraction was 88% at the highest tested concentration. However, since a positive result was observed, this is not considered to have substantially impacted results (i.e. there is not a question of whether negative results were due to concentrations not being high enough).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to this study type. Cell transformation assays may be conducted in the presence of activation, but is not a requirement by study type.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (BALB/c-3T3 cells) and descriptive information (origin = NCI; taken from stock and not maintained beyond first passage) were reported, and the test model is routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	The total plates per dose group for TCE was 12-16. (Reference to duplicate plates is in regards to cell counts for the cytotoxicity assessment.) This is considered appropriate for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology used addressed the intended outcomes of interest (foci with Type III characteristics).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups (approximately 30 days after exposure).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this study type (all foci were scored).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not mentioned in the study report; therefore; this metric is considered not applicable to this study type.

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Study Citation:	A. S. Tu, T. A. Murray, K. M. Hatch, A. Sivak, H. A. Milman (1985). In vitro transformation of BALB/c-3T3 cells by chlorinated ethanes and ethylenes Cancer Letters, 28(1,1), 85-92
Data Type:	In vitro transformation assay for TCE
HERO ID:	17978

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among study group parameters (e.g., test substance, cells used) that could influence the outcome assessment.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variable unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	High	× 1	1	Statistical significance is referenced in the discussion of results for another test compound, but no details regarding the type of statistical test conducted were included. However, data were sufficient data to conduct an independent statistical analysis (based on mean numbers of type III foci/plate and plates with Type III foci/total plates).
	Metric 23: Data Interpretation	Medium	× 2	4	The study authors reported the scoring criteria (characteristics of scored Type III foci) for the test. These characteristics, which were consistent with established practices, were partially cited to another publication (Reznikoff et al., 1973).
	Metric 24: Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints were defined and methods of measurement were partially reported, but the omissions are unlikely to have substantial impact on study results.
	Metric 25: Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.
Overall Quality Determination‡		High		1.2	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 80: **In vitro evaluation results for Rossi et al 1983 for forward mutation study in S. pombe**

Study Citation:	A. M. Rossi, L. Migliore, R. Barale, N. Loprieno (1983). In vivo and in vitro mutagenicity studies of a possible carcinogen, trichloroethylene, and its two stabilizers, epichlorohydrin and 1,2-epoxybutane Teratogenesis, Carcinogenesis, and Mutagenesis, 3(1,1), 75-87
Data Type:	forward mutation in yeast (Schizosuccharomyces pombe)
HERO ID:	18895

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was reported as TCE pure grade
	Metric 2:	Test Substance Source	High	× 1	1	No source was reported. Identity was verified by GC
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was reported 99.98%. impurities were identified and reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Untreated control was reported
	Metric 5:	Positive Controls	Low	× 2	6	No positive control was reported for the in vitro experiment; however, MMS and NMDA were used as positive controls for the in vivo (host-mediated assay).
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were reported and appropriate for the study type
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported. Storage conditions were not reported
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Administration was consistent across dose groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported in table 2 0, 0.22, 2.2, 22.0 mM: 4 doses were reported compared to the recommended 5.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was clearly stated and appropriate for the endpoint.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Number of doses were reported (table 2). Cytotoxicity or range finding assays were not reported and spacing was not justified in the text
	Metric 13:	Metabolic Activation	High	× 1	1	Trials were run with and without metabolic activation. Preparation of S9 was reported.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was reported and is appropriate.
	Metric 15:	Number per Group	Medium	× 1	2	The number of cells/culture was reported and was appropriate for the study type. The number of replicates was not reported.
Domain 5: Outcome Assessment						

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Study Citation:	A. M. Rossi, L. Migliore, R. Barale, N. Loprieno (1983). In vivo and in vitro mutagenicity studies of a possible carcinogen, trichloroethylene, and its two stabilizers, epichlorohydrin and 1,2-epoxybutane Teratogenesis, Carcinogenesis, and Mutagenesis, 3(1,1), 75-87
Data Type:	forward mutation in yeast (Schizosuccharomyces pombe)
HERO ID:	18895

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology addressed the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment protocol was consistent across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each study group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported for each group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was not conducted, however sufficient data were provided to allow for statistical testing.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria was described and were consistent with established criteria
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity was not reported.
	Metric 25:	Reporting of Data	High	× 2	2	Data is reported in tables (table2) by exposure group
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 81: **In vitro evaluation results of Haworth et al 1983 for bacterial reverse mutation study**

Study Citation:	S. Haworth, T. Lawlor, K. Mortelmans, W. Speck, E. Zeiger (1983). Salmonella mutagenicity test results for 250 chemicals Environmental Mutagenesis, 5(Suppl 1,Suppl 1), 3-142
Data Type:	Bacterial reverse mutation for chloral hydrate
HERO ID:	28947

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate with the correct CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported, including manufacturer lot number.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be 99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (water).
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were tested concurrently with each test substance. The identity of each positive control was reported and appropriate for different strains with and without metabolic activation. Positive controls yielded positive results.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described in detail and were applicable to the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration for the pre-incubation protocol was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The maximum dose was chosen based on solubility limits or cytotoxicity. The number of exposure groups and dose spacing was reported and appropriate for this assay (100, 333, 1000, 3333, 4000, 5000, 6667, 7500, or 10000 µg/plate).
	Metric 13:	Metabolic Activation	Medium	× 1	2	The source and method of preparation of the rat liver S9 fraction was reported; however, the concentration of S9 in the bacterial mutagenicity assay was not specified.

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Study Citation: S. Haworth, T. Lawlor, K. Mortelmans, W. Speck, E. Zeiger (1983). Salmonella mutagenicity test results for 250 chemicals Environmental Mutagenesis, 5(Suppl 1,Suppl 1), 3-142
Data Type: Bacterial reverse mutation for chloral hydrate
HERO ID: 28947

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity and donor source of the bacterial strains used here were identified, and these strains are routinely used for the outcome of interest. It was noted that the cultures were "routinely checked for genetic integrity as recommended by Ames et al. (1975)."
	Metric 15:	Number per Group	High	× 1	1	Each assay was plated in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Number of colonies is an objective outcome and blinding assessors is not necessary; however, the identity of each test substance assessed in this study was coded and not known to the assessors.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	A positive result was defined as a "reproducible, dose-related increase, whether it be twofold over background or not." Therefore, no statistical analysis was reported directly in the study; however, this is appropriate for this study design. Raw data are provided and could be analyzed independently.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of colonies) was reported and consistent with current standards.
	Metric 24:	Cytotoxicity Data	High	× 1	1	A dose-setting experiment was conducted to assess cytotoxicity levels (viability, reduced numbers of colonies). If toxicity was observed in the preliminary experiment, the doses for the mutagenicity assay were selected so that the highest dose exhibited some degree of toxicity.

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Study Citation:	S. Haworth, T. Lawlor, K. Mortelmans, W. Speck, E. Zeiger (1983). Salmonella mutagenicity test results for 250 chemicals Environmental Mutagenesis, 5(Suppl 1,Suppl 1), 3-142
Data Type:	Bacterial reverse mutation for chloral hydrate
HERO ID:	28947

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	All data are adequately reported.
Overall Quality Determination‡		High		1.2	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 82: **In vitro evaluation results for Henschler et al 1977 for bacterial reverse mutation study**

Study Citation:	D. Henschler, E. Eder, T. Neudecker, M. Metzler (1977). Carcinogenicity of trichloroethylene: Fact or artifact? Archives of Toxicology, 37(3,3), 233-236
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	29440

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance (used in a previous carcinogenicity assay) was reportedly the Trichloroethylene Toxicology Subcommittee of the Manufacturing Chemists Associations, USA. The identity of the test substance was verified by analytical methods.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The test substance was analyzed using GC-MS Since the identified impurities were = 0.65%, the purity of the test substance was presumably 99.35%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The negative control condition was unclear (presumably untreated). The rate of spontaneous mutations (in the presence or absence of activation) was reported in the legend for Figure 1.
	Metric 5:	Positive Controls	Not Rated	NA	NA	The study did not report using a positive control; however, chemicals used in the assay (including some that suspected carcinogens) elicited positive responses.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were generally described in the legend for Figure 1 with some missing details (e.g., type of media, cell density), cited to another publication (Ames et al. 1973), and appeared to be appropriate for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage was not reported (it was indicated only that test substances were added to the top agar).
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	It is inferred from the information provided that exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses in uL/mL were shown in Figure 1.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported in the legend for Figure 1.

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Study Citation:	D. Henschler, E. Eder, T. Neudecker, M. Metzler (1977). Carcinogenicity of trichloroethylene: Fact or artifact? Archives of Toxicology, 37(3,3), 233-236
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	29440

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of groups was reported (could be determined from Figure 1). A rationale for dose selection was not provided (although a previous carcinogenicity assay was mentioned); it is not clear if doses were high enough to elicit a response.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The presence of a commonly used metabolic activation system (Aroclor 1254-induced rat liver cells) was reported in the legend for Figure 1. Although some details were not described, these omissions are unlikely to have a substantial impact on the results.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model (Salmonella typhimurium strain TA 100) was reported and is routinely used for this study type. The study indicated that results shown were for the "most sensitive strain used, (TA 100)" presumably other S. typhimurium strains were used (but not specified). The source of the strains was not explicitly specified.
	Metric 15:	Number per Group	Medium	× 1	2	The study indicated triplicate plates were used.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology addressed the outcome of interest. It was unclear if mutation frequency was evaluated only in the absence of cytotoxicity.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no reported confounding variables in the test design or procedures.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables unrelated to the test substance were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	The study did not report using statistical analyses, and data provided were not amenable to independent analyses. However, statistical analysis is not required by study type.

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Study Citation:	D. Henschler, E. Eder, T. Neudecker, M. Metzler (1977). Carcinogenicity of trichloroethylene: Fact or artifact? Archives of Toxicology, 37(3,3), 233-236
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	29440

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	Low	× 2	6	The threshold for a positive response was not reported.
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.
	Metric 25:	Reporting of Data	Low	× 2	6	Data were reported quantitatively (Figure 1) for all exposure groups in S typhimurium strain TA 100 only. Data for other strains (presumably tested in the study) were not shown or described qualitatively.
Overall Quality Determination‡			Unacceptable⋆⋆		2.3	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 83: **In vitro evaluation results for Price et al 1978 for cell transformation assay in rat embryo cells**

Study Citation:	P. J. Price, C. M. Hassett, J. I. Mansfield (1978). Transforming activities of trichloroethylene and proposed industrial alternatives In Vitro, 14(3,3), 290-293					
Data Type:	Cell transformation assay for TCE					
HERO ID:	29449					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name (trichloroethylene; TCE).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified (Fisher Scientific) and catalog and lot numbers were provided (Catalog No. T-341, Lot No. 754766).
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was such that any observed effects were highly likely to be due to the nominal test substance itself. The test substance was tested for purity using American Chemical Society specifications; the resultant purity was > 99.9%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using a concurrent negative control group, but all conditions were not equal to those of treated groups. However, the identified differences are considered to be minor limitations that are unlikely to have substantial impact on results. It is indicated that the negative control was acetone at a concentration of 1:1000; the positive control was also diluted in acetone. The study does not state that the test substance was diluted in acetone. However, an additional medium only group was used.
	Metric 5:	Positive Controls	Medium	× 2	4	A concurrent positive control was used, and is appropriate for the study type (i.e., cell transformation assays). The results indicate that the positive control induced transformation; however, the response not further characterized, and appeared to be similar in magnitude to the response for the test substance(s).
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures (e.g., test conditions, cell density, culture media, and volumes) were described in adequate detail.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type (no established criteria for this study type).
Domain 3: Exposure Characterization						

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Study Citation:	P. J. Price, C. M. Hassett, J. I. Mansfield (1978). Transforming activities of trichloroethylene and proposed industrial alternatives In Vitro, 14(3,3), 290-293
Data Type:	Cell transformation assay for TCE
HERO ID:	29449

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation/storage conditions were not described in detail (other than the test substance has a half-life > 2 years); however, this would not be expected to have a substantial impact on the results given that it is a short-term study.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (48 hours) was reported and appears to be appropriate for the study type/outcome of interest (cell transformation).
	Metric 12:	Exposure Route and Method	Low	× 1	3	There were deficiencies regarding the number of exposure groups and/or concentration spacing. Only two concentrations of the test substance were tested (with no rationale for their selection), and the response between the two exposure groups was nearly indistinguighable.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to this study type. Cell transformation assays may be conducted in the presence of activation, but is likely not a requirement by study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was reported along with limited descriptive information (described previously in Freeman et al. 1975). Limited information regarding the cells (passage, genetic information) was provided. The source was not reported. It is not clear that this cell type (Fischer rat embryo F1706 cells) are routinely used for this study type.
	Metric 15:	Number per Group	High	× 1	1	For the transformation assay, the use of quadruplicate cultures were reported. The number of replicates per study group were reported and were considered appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	It was not clear that the outcome assessment (evidence of transformation 4 subcultures after treatment) was a sensitive measure of transformation potential. Mean numbers of foci (for three dishes) also did not show an exposure-related response pattern.

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Study Citation: P. J. Price, C. M. Hassett, J. I. Mansfield (1978). Transforming activities of trichloroethylene and proposed industrial alternatives In Vitro, 14(3,3), 290-293
Data Type: Cell transformation assay for TCE
HERO ID: 29449

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not mentioned in the study report; therefore; this metric is considered not applicable to this study type. The study indicates that morphological transformation was based on the first observation of foci formation (foci with cells lacking contact inhibition and orientation; growth of macroscopic foci on semisolid agar).
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among study group parameters (e.g., test substance, cells used) that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variable unrelated to exposure were reported or identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Unacceptable	× 1	4	No statistical analyses were conducted (cell transformation assay) and data for average number of foci (three plates) were not provided with a measure of variation (for independent analyses). The number of plates with foci/number of plates were also not reported/could not be analyzed. There was no evidence that the positive control induced a statistically significantly increased transformation frequency.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were partially reported (e.g., characteristics of transformed foci). However, a complete description of the criteria for a positive response was not adequately described (transformation by the fourth subsculture and/or numbers of microscopic foci).
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints were defined and methods of measurement were partially reported. The authors indicated that a test was conducted before the transformation assay. TCE was tested only at nontoxic concentrations (relative plating efficiencies of 84% and 97%).
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.

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Study Citation:	P. J. Price, C. M. Hassett, J. I. Mansfield (1978). Transforming activities of trichloroethylene and proposed industrial alternatives In Vitro, 14(3,3), 290-293
Data Type:	Cell transformation assay for TCE
HERO ID:	29449

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		Unacceptable⋆⋆		1.7	
Extracted		No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 84: **In vitro evaluation results for Price et al 1978 for cell transformation assay in rat embryo cells**

Study Citation:	P. J. Price, C. M. Hassett, J. I. Mansfield (1978). Transforming activities of trichloroethylene and proposed industrial alternatives In Vitro, 14(3,3), 290-293
Data Type:	Cell transformation assay for perc
HERO ID:	29449

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name (tetrachloroethylene; TTCl).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified (Eastman Kodak). Although batch/lot numbers were not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using a concurrent negative control group, but all conditions were not equal to those of treated groups. However, the identified differences are considered to be minor limitations that are unlikely to have substantial impact on results. It is indicated that the negative control was acetone at a concentration of 1:1000; the positive control was also diluted in acetone. The study does not state that the test substance was diluted in acetone. However, an additional medium only group was used.
	Metric 5:	Positive Controls	Medium	× 2	4	A concurrent positive control was used, and is appropriate for the study type (i.e., cell transformation assays). The results indicate that the positive control induced transformation; however, the response not further characterized, and appeared to be similar in magnitude to the response for the test substance(s).
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures (e.g., test conditions, cell density, culture media, and volumes) were described in adequate detail.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation/storage conditions were not described in detail (other than the test substance has a half-life > 2 years); however, this would not be expected to have a substantial impact on the results given that it is a short-term study.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.

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Study Citation:	P. J. Price, C. M. Hassett, J. I. Mansfield (1978). Transforming activities of trichloroethylene and proposed industrial alternatives In Vitro, 14(3,3), 290-293
Data Type:	Cell transformation assay for perc
HERO ID:	29449

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (48 hours) was reported and appears to be appropriate for the study type/outcome of interest (cell transformation).
	Metric 12:	Exposure Route and Method	Low	× 1	3	There were deficiencies regarding the number of exposure groups and/or concentration spacing. Only two concentrations of the test substance were tested (with no rationale for their selection).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to this study type. Cell transformation assays may be conducted in the presence of activation, but is not a requirement by study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was reported along with limited descriptive information (described previously in Freeman et al. 1975). Limited information regarding the cells (passage, genetic information) was provided. The source was not reported. It is not clear that this cell type (Fischer rat embryo F1706 cells) is routinely used for this study type.
	Metric 15:	Number per Group	High	× 1	1	For the transformation assay, the use of quadruplicate cultures were reported. The number of replicates per study group were reported and were considered appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	It was not clear that the outcome assessment (evidence of transformation 2 to 4 subcultures after treatment) was a sensitive measure of transformation potential.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not mentioned in the study report; therefore; this metric is considered not applicable to this study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among study group parameters (e.g., test substance, cells used) that could influence the outcome assessment.

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Study Citation:	P. J. Price, C. M. Hassett, J. I. Mansfield (1978). Transforming activities of trichloroethylene and proposed industrial alternatives In Vitro, 14(3,3), 290-293
Data Type:	Cell transformation assay for perc
HERO ID:	29449

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variable unrelated to exposure were reported or identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Unacceptable	× 1	4	No statistical analyses were conducted (cell transformation assay) and data for average number of foci (three plates) were not provided with a measure of variation (for independent analyses). The number of plates with foci/number of plates were also not reported/could not be analyzed. There was no evidence that the positive control induced a statistically significantly increased transformation frequency.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were partially reported (e.g., characteristics of transformed foci). However, a complete description of the criteria for a positive response was not provided (transformation by the a certain subsculture and/or numbers of microscopic foci).
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints were defined and methods of measurement were partially reported. The authors indicated that a test was conducted before the transformation assay. Perc was tested only at concentrations that yielded relative plating efficiencies of 88% and 63%.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.
Overall Quality Determination‡			Unacceptable⋆⋆		1.8	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 85: **In vitro evaluation results of Simmon et al 1977 for bacterial reverse mutation**

Study Citation:	V. F. Simmon, K. Kauhanen, R. G. Tardiff (1977). Mutagenic activity of chemicals identified in drinking water Developments in Toxicology and Environmental Science, 2, 249 249-258					
Data Type:	Bacterial reverse mutation for TCE					
HERO ID:	29451					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name (1,1,2-trichloroethylene).
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported incompletely (reported as a commercial supplier). Since the test substance is not expected to vary in composition and the test substance was obtained from a commercial supplier, the omitted details are unlikely to have a substantial impact on the results.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity and grade of test substance were not reported ("reagents of the highest available purity"). It was indicated that purity was not determined for most chemicals. This is not expected to have impacted results, as the test substance was obtained from a commercial supplier.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	A concurrent negative control was used; the control response was shown graphically in Figure 22 (for the bacterial assay in dessicators). The response was not reported/shown for the assay in Saccharomyces cerevisiae.
	Metric 5:	Positive Controls	Low	× 2	6	A concurrent positive control was used (unnamed), but the control response was not described.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and cited in another publication (Ames et al. 1975), but appeared to be appropriate for the assays in desiccators (bacteria) and in suspension (yeast); some details (e.g., cell density for the bacterial assay) were reported incompletely. Special test conditions were used to account for the volatility of the test substance.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance preparation was reported (e.g., test chemical added to glass petri plate for bacterial assay); lack of storage conditions are not likely to substantially impact the study results given the study design (single-dose administration).

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Study Citation:	V. F. Simmon, K. Kauhanen, R. G. Tardiff (1977). Mutagenic activity of chemicals identified in drinking water Developments in Toxicology and Environmental Science, 2, 249 249-258
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	29451

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were reported or inferred from the text with few study details (or cited to Ames et al. 1975). Exposures were reportedly for "7 to 10 hours" (unclear if time varied among concentrations or different chemicals tested); however, these differences were not expected to substantially affect the study results.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The amount of test substance used in the bacterial assay was presented graphically in the study report (reported in uL/plate).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	Exposure duration was reported (7-10 hours for the bacterial assay, 4 hours for the yeast assay). The duration of these assays was presumed appropriate for the study type (given that positive responses were observed for the test chemical or other chemicals used in the study). It is possible that the exposure duration for the bacterial assay (7 to 10 hours) varied across dose levels. It is also possible that the variation in exposure duration was across test substances instead, so this metric is still considered acceptable.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups was reported (e.g., 5 doses based on data presented graphically in Figure 22). The number of exposure groups and concentration spacing were justified by study authors (based on study type and cytotoxicity studies) and considered adequate to address the purpose of the study. The study states (for all chemicals) that a wide range of doses were tested up to a given concentration or until a toxic concentration was achieved (whichever was lower).
	Metric 13:	Metabolic Activation	Medium	× 1	2	The presence of a commonly used metabolic activation system (e.g., rat or mice liver cells cited to Ames et al. 1975) was reported in the study; however, some details were not described. These omissions are unlikely to have a substantial impact on the results.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported along with limited descriptive information. The test model was routinely used for the outcome of interest. Reporting limitations are unlikely to have a substantial impact on results.

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Study Citation:	V. F. Simmon, K. Kauhanen, R. G. Tardiff (1977). Mutagenic activity of chemicals identified in drinking water Developments in Toxicology and Environmental Science, 2, 249 249-258
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	29451

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Low	× 1	3	The number of replicates per study group were not reported (though procedures were reportedly consistent with Ames et al. 1975). Because there are no error bars in any graphs, it is considered likely that only one plate per dose was included in the study design, which is lacking.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcome(s) of interest and was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	It is not clear that the exposure duration (7-10 hr) and post-exposure incubation time ("approximately" 40 hrs) were equal for all doses of a test substance.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among study group parameters (e.g., test substance, cells used) identified that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variable unrelated to exposure were reported or identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analysis was not conducted, and insufficient information was provided for independent statistical analysis (standard deviation and number of replicates not provided), likely due to only one replicate per dose level being included in the study design. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay.
	Metric 23:	Data Interpretation	Low	× 2	6	Scoring and/or evaluation criteria were not reported and the omissions are likely to have a substantial impact on interpretation of the results.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were evaluated, but the methods of measurements were not fully described or reported.

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Study Citation:	V. F. Simmon, K. Kauhanen, R. G. Tardiff (1977). Mutagenic activity of chemicals identified in drinking water Developments in Toxicology and Environmental Science, 2, 249 249-258
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	29451

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	Low	× 2	6	Data were presented adequately for the desiccator assay. It was unclear whether TCE was tested in the other assays (e.g. yeast) described in the study report; if so, data reporting for these assays is inadequate.
Overall Quality Determination‡		Medium		2.0	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 86: **In vitro evaluation results for Kringstad et al 1981 for mutation assay in S. typhimurium**

Study Citation:	K. P. Kringstad, P. O. Ljungquist, F. de Sousa, L. M. Stromberg (1981). Identification and mutagenic properties of some chlorinated aliphatic compounds in the spent liquor from kraft pulp chlorination Environmental Science and Technology, 15(5,5), 562-566
Data Type:	in vitro mutation assay in S. typhimurium - TCE
HERO ID:	35086

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (E. Merck). The product number and batch/lot number were not reported; however, the material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity and/or grade of the test substance was reported (99.5%)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors report using a vehicle control (ether)
	Metric 5:	Positive Controls	Low	× 2	6	A positive control was used (methyl methanesulfonate; however, the response of the positive control were not reported.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were briefly described, but appeared appropriate. More detailed methods were cited to other references (Ander et al., 1977).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was described as added in ether solution (20ul/plate).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were reported to be administered consistently across treated and control groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	One test concentration was reported in the results without ambiguity (0.1 mg/plate)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	The exposure duration was not reported. More detailed methods were cited to other references (Ander et al., 1977).

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Study Citation:	K. P. Kringstad, P. O. Ljungquist, F. de Sousa, L. M. Stromberg (1981). Identification and mutagenic properties of some chlorinated aliphatic compounds in the spent liquor from kraft pulp chlorination Environmental Science and Technology, 15(5,5), 562-566
Data Type:	in vitro mutation assay in S. typhimurium - TCE
HERO ID:	35086

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure concentrations were not clearly reported. The study noted that the amount of single model compounds added was varied over a wide range covering survival from 1-100%, including 6-8 different (unspecified) dosage levels. Only 1 test concentration was reported in the results. There is no indication if there was toxicity at the highest dose tested. It is noted in the results that the doses presented "were about the highest possible which yield 70-100% bacterial survival for each tested compound". This metric is determined to be unacceptable due to the uncertainty of cytotoxicity at this dose.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable; the test organism, S. typhimurium was used without the addition of metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test models and source were reported and appropriate for the outcome of interest (S. typhimurium TA 1535). It is noted that it is unusual to only utilize one S. typhmurium tester strain for the bacterial reverse mutation assay; however, the single strain utilized is considered valid in itself.
	Metric 15:	Number per Group	Medium	× 1	2	Reported results were mean values of 3 or more assays. There is some uncertainty because the minimum number of replicates was reported, but the specific amount of replicates for each treatment group was not reported. However, 3 assays is considered sufficient for the outcome of interest.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across the controls and treated groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This method is not applicable to the outcome.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no confounding variables noted in the study

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Study Citation:	K. P. Kringstad, P. O. Ljungquist, F. de Sousa, L. M. Stromberg (1981). Identification and mutagenic properties of some chlorinated aliphatic compounds in the spent liquor from kraft pulp chlorination Environmental Science and Technology, 15(5,5), 562-566
Data Type:	in vitro mutation assay in S. typhimurium - TCE
HERO ID:	35086

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variable unrelated to exposure were reported or identified
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistics were not used to assess increased revertants/plate from the control. It was noted that the compound was listed positive when the number of revertants exceeded the background level by a factor of 2 or more. Only means (with no measure of variance, e.g. standard deviation; and no specific number of replicates) were included in the results so independent statistical analysis could not be performed. Statistical analysis is not necessarily required for the bacterial reverse mutation assay, so the data analysis is considered acceptable.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria were reported and appropriate.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoints and methods were described (cell death)
	Metric 25:	Reporting of Data	Low	× 2	6	Data for the outcome was presented; however, data were not shown for each study group, data for the positive control and cytotoxicity data were not reported.
Overall Quality Determination‡			Unacceptable⋆⋆		1.5	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 87: **In vitro evaluation results of Nakamura et al 1987 for DNA repair**

Study Citation:	S. I. Nakamura, Y. Oda, T. Shimada, I. Oki, K. Sugimoto (1987). SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: Examination of 151 chemicals Mutation Research Letters, 192(4,4), 239-246
Data Type:	DNA repair for TCE
HERO ID:	51515

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as trichloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (Wako pure chemical). Although a batch/lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity/grade of the test substance was not reported. However, it was indicated that chemicals were of the highest quality commercially available.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative controls were reported; however, it is not clear if they were run concurrently with test substance (e.g., DMSO was one of the 151 chemicals tested in the assay).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive responses were observed for several of the 151 chemicals tested in this study (demonstrating that the test is capable of detecting a positive response) it is unclear if these were run concurrently with test substance. It is noted the list of chemicals tested included test substances used as positive controls in the Ames assay.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were briefly described and partially cited to another publication (Oda et al 1985).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Based on text and properties of test substance we can conclude test substance was prepared in DMSO, although not explicity stated. Storage conditions were not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Since responses were negative for all doses tested, only the highest dose was reported (1950 ug/mL).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (2 hours) and appropriate for the outcome of interest.

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Study Citation:	S. I. Nakamura, Y. Oda, T. Shimada, I. Oki, K. Sugimoto (1987). SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: Examination of 151 chemicals Mutation Research Letters, 192(4,4), 239-246
Data Type:	DNA repair for TCE
HERO ID:	51515

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups and/or spacing was not reported. Only the highest tested dose was reported (no rationale provided).
	Metric 13:	Metabolic Activation	Medium	× 1	2	Method of preparing liver S9 fraction are only partially reported (i.e., prepared from rats pretreated with phenobarbital and 5,6-benzoflavone).
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The cell line was developed in house; limited details were provided. The system was described as novel (not yet routinely used to assess this outcome).
	Metric 15:	Number per Group	Low	× 1	3	The number of replicates per group was not indicated.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Methods for outcome assessment were largely cited to another publication (Miller, 1972).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Binding was not necessary for this study.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variable were reported in test design/procedure.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Data are presented qualitatively (i.e., reported as negative). Statistical analyses do not appear to have been performed (despite the use of the term 'significant' in the results section), but are not required by study type (fold changes can be used to evaluate the response).
	Metric 23:	Data Interpretation	High	× 2	2	It was indicated that a 2-fold increase in beta-galactosidase activity above background levels was considered a positive effect. The study authors further classified chemicals used in the study as potent inducers (6-fold changes), intermediate inducers (3-fold changes), or weak inducers (2-fold changes).

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Study Citation:	S. I. Nakamura, Y. Oda, T. Shimada, I. Oki, K. Sugimoto (1987). SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: Examination of 151 chemicals Mutation Research Letters, 192(4,4), 239-246
Data Type:	DNA repair for TCE
HERO ID:	51515

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.
	Metric 25: Reporting of Data	Medium	× 2	4	Only one data point is reported (highest tested concentration); however, since the results were negative, this is unlikely to have a substantial impact on results.
Overall Quality Determination‡		Unacceptable⋆⋆		1.9	
Extracted		No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 88: **In vitro evaluation results for Greim et al 1975 for bacterial mutagenicity study**

Study Citation:	H. Greim, G. Bonse, Z. Radwan, D. Reichert, D. Henschler (1975). Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation Biochemical Pharmacology, 24(21,21), 2013-2017
Data Type:	Mutagenicity of E. coli - TCE
HERO ID:	58073

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Trichloroethylene was identified by chemical name and structure (Table 1).
	Metric 2:	Test Substance Source	High	× 1	1	Obtained from Merc & Co., Darmstadt.
	Metric 3:	Test Substance Purity	High	× 1	1	Chemicals from this source were obtained as a.g. reagents.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	The study authors did not report the use of a concurrent negative control group.
	Metric 5:	Positive Controls	Medium	× 2	4	A positive control group was not reported, but vinyl chloride was concurrently tested and the authors reported it produced positive responses with metabolic activation, indicating the test system was capable of detecting a positive response (although the evaluation criteria for a positive response was not specified).
	Metric 6:	Assay Procedures	Medium	× 1	2	Test methods/procedures were briefly described or were cited to another source (C. Mohn, et al. 1974), but appeared appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The study only reports varying concentrations of 5 uL of the liquid test substance were added (injected) to the medium. No other preparation details were provided. The pre-incubation method was used and appropriate for the test substances. No storage details were required due to the short study duration (2 hours).
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposure appears consistent across the study groups; however, it is not specifically stated. Methods were briefly described or cited elsewhere.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Trichloroethylene was tested at 3.3 nM.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was 2 hours and was appropriate for this study type.

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Study Citation: H. Greim, G. Bonse, Z. Radwan, D. Reichert, D. Henschler (1975). Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation Biochemical Pharmacology, 24(21,21), 2013-2017
Data Type: Mutagenicity of E. coli - TCE
HERO ID: 58073

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	One concentration was used on one bacterial strain (E. coli K12) with 4 different operons (gal+, arg+, MTR, and nad+). Cell survival was 76% for trichloroethylene. the study notes that the test concentrations were chosen based on the results of a preliminary experiment in order to not reduce cell survival by >20%. No additional details of the preliminary experiment results were provided.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The study reports cells were exposed both with and without metabolic activation. 5 mg of liver microsomes from male mice pretreated with 0.1% phenobarbital in drinking water for 10 days were used as the metabolic activation. Method of preparation was not reported.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	E. coli K12 was used in this experiment with 4 different operons (gal+, arg+, MTR, and nad+). It is unclear if this strain was from a commercial source or laboratory-maintained. No other strains were tested in a mutagenicity test.
	Metric 15:	Number per Group	Low	× 1	3	The number of replicates used in this study was not specified, but it is assumed as a single assay.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Mutagenicity was evaluated by counting the number of colony-forming units on the selective media per the number of colony-forming units on the complete medium, presented as the % spontaneous mutation rate (Table 1). Cytotoxic concentrations were deliberately avoided based on the results of the preliminary test.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	No inconsistencies were reported, and consistency appeared appropriate. However, details results in the absence of metabolic activation were not provided.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design (mutagenicity assay).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design, as no subjective outcomes were assessed.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each study replicate or group.

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Study Citation:	H. Greim, G. Bonse, Z. Radwan, D. Reichert, D. Henschler (1975). Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation Biochemical Pharmacology, 24(21,21), 2013-2017
Data Type:	Mutagenicity of E. coli - TCE
HERO ID:	58073

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analysis was not performed, and although individual results were provided in Table 1 in the presence of metabolic activation, no negative control was used and a dose-response analysis is not possible because only 1 concentration was tested. Results in the absence of metabolic activation were generally summarized as negative and no individual data was provided. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay.
	Metric 23:	Data Interpretation	Low	× 2	6	The scoring and/or evaluation criteria was not described, and it is unclear how a positive result was determined.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The percent survival of bacteria on the full media was reported, and the chosen concentration was based on the cytotoxicity results from a preliminary test, with a goal of <20% cell death.
	Metric 25:	Reporting of Data	High	× 2	2	Individual results were reported for in Table 1 in the presence of metabolic activation. All chemicals tested (6 total) were reported as negative for mutagenicity in the absence of metabolic activation (individual results not reported).
Overall Quality Determination‡			Unacceptable⋆⋆		2.0	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 89: **In vitro evaluation results for Kline et al 1982 for bacterial mutagenicity study**

Study Citation:	S. A. Kline, E. C. Mccoy, H. S. Rosenkranz, B. L. Van Duuren (1982). Mutagenicity of chloroalkene epoxides in bacterial systems Mutation Research, 101(2,2), 115-125
Data Type:	in vitro mutation assay in S. typhimurium and E. coli - TCEoxide
HERO ID:	58237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene oxide (chemical structure provided)
	Metric 2:	Test Substance Source	Unacceptable	× 1	4	Analytical verification of the synthesized test substance was not conducted.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade of the test substance was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors report using both untreated and vehicle controls (acetone).
	Metric 5:	Positive Controls	High	× 2	2	Appropriate positive controls were used (AF-2 for E.coli and NaN3 for S. typhimurium) in the mutagenicity assay.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were briefly described but appeared appropriate. More detailed methods were cited to other references (McCoy et al., 1978 for mutagenicity assay and Hyman et al., 1980 for the DNA-repair assay).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Test substance preparation was described as diluted in acetone (10ul dilutions); The storage of the test substance was not reported. This is likely to have affected results, given that the half life of trichloroethylene-oxide was reported to be 90 seconds in water. It is likely that the lack of reported test substance storage substantially affected results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were reported to be administered consistently across treated and control groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentration was reported in the results without ambiguity Mutagenicity TCE-oxide (5, 2.5, 1.3, 0.5, 0.25 mM)

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Study Citation:	S. A. Kline, E. C. Mccoy, H. S. Rosenkranz, B. L. Van Duuren (1982). Mutagenicity of chloroalkene epoxides in bacterial systems Mutation Research, 101(2,2), 115-125
Data Type:	in vitro mutation assay in S. typhimurium and E. coli - TCEoxide
HERO ID:	58237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration was reported (20 minutes). It is noted that given a half life of 1.5 minutes, it would be expected that 0.0097% of the original amount of the test substance would be present in solution after 20 minutes.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure concentrations were reported. The number of exposure groups and spacing of exposure levels were not justified. No effects were observed with this test substance in the bacterial reverse mutation assay. Additionally, no cytotoxicity was observed at the higher doses of the test substance; therefore, it is not clear that the highest dose tested was high enough.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable; the test organism, TCE and Perc metabolites were tested without the addition of metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test models were reported with some descriptive information and appropriate for the outcome of interest; The source of the bacteria was not reported Mutation assay: S. typhimurium 1535 and E. coli WP2uvrA
	Metric 15:	Number per Group	High	× 1	1	3 replicates per treatment group is considered adequate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across the controls and treated groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This method is not applicable to the outcome.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial strain/batch/lot number of organisms or models used per group, size, and/or quality of tissues exposed was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						

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Study Citation:	S. A. Kline, E. C. Mccoy, H. S. Rosenkranz, B. L. Van Duuren (1982). Mutagenicity of chloroalkene epoxides in bacterial systems Mutation Research, 101(2,2), 115-125
Data Type:	in vitro mutation assay in S. typhimurium and E. coli - TCEoxide
HERO ID:	58237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Medium	× 1	2	Statistics were not used to assess increased revertants/plate from the control. Means (with standard deviation) were included in the results so independent statistical analysis may be performed. Statistical analysis is not necessarily required for the bacterial reverse mutation assay, so the data analysis is considered acceptable.
	Metric 23:	Data Interpretation	Low	× 2	6	The evaluation criteria were reported to be exhibiting toxicity, as evidenced by a decrease in the spontaneous frequency of the revertants and/or by an inhibition of the growth of the bacteria; evaluation of mutagenic potential was not described.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoints were described (decreased spontaneous frequency of revertants)
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcomes were presented for each exposure groups, including negative and positive controls
Overall Quality Determination‡			Unacceptable⋆⋆		1.8	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 90: **In vitro evaluation results for Kline et al 1982 for bacterial DNA repair study**

Study Citation:	S. A. Kline, E. C. Mccoy, H. S. Rosenkranz, B. L. Van Duuren (1982). Mutagenicity of chloroalkene epoxides in bacterial systems Mutation Research, 101(2,2), 115-125
Data Type:	DNA-repair assay in E. coli - TCEoxide
HERO ID:	58237

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene oxide (chemical structure provided)
	Metric 2:	Test Substance Source	Unacceptable	× 1	4	Analytical verification of the synthesized test substance was not conducted.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade of the test substance was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors report using a vehicle control (acetone).
	Metric 5:	Positive Controls	High	× 2	2	An appropriate positive control was used (ethyl methanesulfonate for the DNA-repair assay).
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were briefly described but appeared appropriate. More detailed methods were cited to other references (McCoy et al., 1978 for mutagenicity assay and Hyman et al., 1980 for the DNA-repair assay).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Test substance preparation was described as diluted in acetone (10ul dilutions); The storage of the test substance was not reported. This is likely to have affected results, given that the half life of trichloroethylene-oxide was reported to be 90 seconds in water. It is likely that the lack of reported test substance storage substantially affected results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were reported to be administered consistently across treated and control groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentration was reported in the results without ambiguity TCE-oxide (0.11, 0.08, 0.06, 0.01, 0.006 uM/ml)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration was reported (20 minutes). It is noted that given a half life of 1.5 minutes, it would be expected that 0.0097% of the original amount of the test substance would be present in solution after 20 minutes.

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Study Citation: S. A. Kline, E. C. Mccoy, H. S. Rosenkranz, B. L. Van Duuren (1982). Mutagenicity of chloroalkene epoxides in bacterial systems Mutation Research, 101(2,2), 115-125
Data Type: DNA-repair assay in E. coli - TCEoxide
HERO ID: 58237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure concentrations were reported. The number of exposure groups and spacing of exposure levels were not justified, but were adequate to show results relevant to the outcome of interest
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable; the test organism, TCE and Perc metabolites were tested without the addition of metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test models were reported with some descriptive information and appropriate for the outcome of interest; The source of the bacteria was not reported DNA-repair assay: E. coli polA1+ and E. coli polA1-
	Metric 15:	Number per Group	Medium	× 1	2	2 replicates per treatment group is considered somewhat lacking.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across the controls and treated groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This method is not applicable to the outcome.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial strain/batch/lot number of organisms or models used per group, size, and/or quality of tissues exposed was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Results for the DNA-repair assay are expressed as % survival compared to control. This was based on an average (of 2 plates) colonies/plate (variance was not reported) for each test concentration. A survival index (% survival polA1+/%survival pol A1+) was also reported. Statistical analysis is not necessarily required for this assay, so the data analysis is considered acceptable.

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Study Citation:	S. A. Kline, E. C. Mccoy, H. S. Rosenkranz, B. L. Van Duuren (1982). Mutagenicity of chloroalkene epoxides in bacterial systems Mutation Research, 101(2,2), 115-125
Data Type:	DNA-repair assay in E. coli - TCEoxide
HERO ID:	58237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria were reported and appropriate (Survival index values below 0.85 indicated preferential inhibition of polA-)
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoints were described (decreased spontaneous frequency of revertants)
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcomes were presented for each exposure groups, including negative and positive controls
Overall Quality Determination‡			Unacceptable⋆⋆		1.7	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 91: **In vitro evaluation results for Waskell 1978 for bacterial reverse mutation study**

Study Citation:	L. Waskell (1978). A study of the mutagenicity of anesthetics and their metabolites Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 57(2,2), 141-153
Data Type:	Bacterial reverse mutation - TCE and metabolites
HERO ID:	58248

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE and metabolites were identified by established nomenclature.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturers were reported. Batch/lot numbers were not given; however, the test materials are not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Study authors acknowledged using a concurrent negative control group, but details regarding the negative control group were not reported. Results were reported as # revertants above control.
	Metric 5:	Positive Controls	High	× 2	2	Concurrent positive controls were reported for the plate incorporation experiment (2-aminofluorene), the closed container study (vinylidene chloride as a volatility control), and the DNA repair assay (2-chloroacetaldehyde, methyl methanesulfonate).
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and also cited in other publications.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage was not provided.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Critical exposure details are not provided; however, other papers are referenced for details on methods.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses/concentrations were reported in tables.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	Details are not provided for exposure duration (except for mutagenicity for volatile compounds in Table 2); however, other papers are referenced for details on methods.

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Study Citation:	L. Waskell (1978). A study of the mutagenicity of anesthetics and their metabolites Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 57(2,2), 141-153
Data Type:	Bacterial reverse mutation - TCE and metabolites
HERO ID:	58248

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	For mutagenicity, 5 concentrations of TCE in the closed container assay; 6 concentrations of chloral hydrate data in plate assay. The number of groups used for other (non-volatile) metabolites (e.g. DCA) used in the plate and/or DNA repair assays was not reported (only the maximum non-toxic quantity tested/plate shown in Table 1 or the quantity tested in mg in Table 3); however, assays were performed according to standard protocols (cited to other publications).
	Metric 13:	Metabolic Activation	Medium	× 1	2	The reverse mutation assay was conducted in the presence and absence of activation (rat liver homogenate). The method of preparation was largely cited to another publication (Ames et al. 1975). The final concentration of liver homogenate was not specified, but these conditions were cited to other references.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test strains used (Salmonella typhimurium strains TA 100, TA 98, and TA 1535 for mutagenicity and the DNA-repair deficient strains) are routinely used for studies of this type. Details about these strains were cited to other publications. It appears that strains were obtained from a laboratory-maintained source.
	Metric 15:	Number per Group	High	× 1	1	Duplicate cultures per strain (mutagenicity assay).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in test design and procedures were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						

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Study Citation:	L. Waskell (1978). A study of the mutagenicity of anesthetics and their metabolites Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 57(2,2), 141-153
Data Type:	Bacterial reverse mutation - TCE and metabolites
HERO ID:	58248

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Unacceptable	× 1	4	Statistical methods were not described with the exception of the legend for Figure 2 (which indicated statistically significant effects based on a Student's t-test in the mutagenicity assay for chloral hydrate). It's unclear if statistics were performed for all groups, and data provided are not suitable for independent statistical analysis.
	Metric 23:	Data Interpretation	Low	× 2	6	The study report did not clearly define the criteria for a positive result. Based on information presented in Figure 2, the dose-responsiveness and/or statistical significance of effects was considered. Mutagenicity was evaluated as the number of revertants greater than controls.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	The study provided results based on maximum non-toxic doses; however, the cytotoxicity endpoint was fully described/reported.
	Metric 25:	Reporting of Data		× 2	NA	Data for mutagenicity were not adequately reported. Negative data were reported as the number of revertants above control levels (raw data for numbers of revertants were not shown).
Overall Quality Determination‡			Unacceptable⋆⋆		2.2	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 92: **In vitro evaluation results for Beliles et al 1980 for unscheduled DNA synthesis study**

Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	PERC UDS
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chemical was identified by name and CAS
	Metric 2:	Test Substance Source	High	× 1	1	Source was reported, North Strong, and analytically verfied
	Metric 3:	Test Substance Purity	Medium	× 1	2	analyzed 91.43% purity, impurities were not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Solvent control was reported
	Metric 5:	Positive Controls	High	× 2	2	MNNG and BaP were reported as positive con trols -/+ S9, respectively.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedure was partially reported and appeared appropriate for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance was prepared in DMSO solvent and cell medium.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was assumed to be consistent across all study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Test concentrations range 0., 0.5, 1.0, 5.015.0 ug/mL (reports ul/ml in results but can be converted).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	Exposure duration was 1.5h, less than recommended but only slightly.
	Metric 12:	Exposure Route and Method	High	× 1	1	Concentrations were 3 doses and controls and spacing was based on cytotoxicity seen at the high dose and appeared to be .
	Metric 13:	Metabolic Activation	High	× 1	1	metabolic activation S9 was reported
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model is reported human diploid WI-38 cells and is appropriate for the study
	Metric 15:	Number per Group	Low	× 1	3	Cell number per group was not reported but was described as confluent
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was adequate for the outcome of interest

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Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	PERC UDS
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Exposure assessment is assumed to be consistent across study groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Cell number counted/slides were not reported but was done with spec and is inferred to be autocounted
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial information was not reported
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analysis was not reported due to lack of replicates
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria was reported as 150% or greater than controls, and appears to be appropriate.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints were previously cited, cell growth, and instances were reported at the high dose.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and doses
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 93: **In vitro evaluation results for Beliles et al 1980 for host-mediated assay in mice**

Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	PERC host mediated assay TA98 in CD-1 mice
HERO ID:	58331

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chemical was identified by name and CAS
	Metric 2:	Test Substance Source	High	× 1	1	Source was reported, North Strong, and analytically verfied
	Metric 3:	Test Substance Purity	Medium	× 1	2	Analyzed 91.43% purity, impurities were not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Filtered air control animals
	Metric 5:	Positive Controls	Low	× 2	6	2-aminoanthracene was used as a positive control specifically for TA98 frameshift, but gives variable results; dimethylnitrosamine was used as a second positive control for TA 1535
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were reported, however the collection of peritoneal fluid from 5 animals was mistakenly pooled, rather than analyzed individually and deviates from standard practice
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Method and equipment used to generate the test substance as a vapor were reported and appropriate.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was assumed to be consistent across all study groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were 100 and 500 ppm
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	Exposure duration of indicator in organism was 3 h following animal exposure (5d)
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Number of exposure groups was reported, 2, and appeared adequate, spacing was not justified
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable to the study type
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was reported, TA98 indicator in CD-1 host, and is routinely used for the outcome of interest.

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Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	PERC host mediated assay TA98 in CD-1 mice
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	Bacterium were cultured to 1x 10ˆ10 cells/ml with 1ml injected and was appropriate for the study
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was adequate for the outcome of interest
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	IP injection time of the indicator into host was not reported and unclear if consistent between groups (but within 2h after exposure)
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Cell number counted/slides were not reported but was done with spec and is inferred to be autocounted
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial information was not reported
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was not reported but data was sufficient for independent analysis
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria was reported as greater than 2 fold the control value and appears appropriate for the study
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	not applicable for the study type
	Metric 25:	Reporting of Data	High	× 2	2	Data is reported qualitatively in table 79 and quantitatively (pooled samples of 5) in table 80
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 94: **In vitro evaluation results for Vamvakas et al 1987 for S. typhimurium mutagenicity study**

Study Citation:	S. Vamvakas, W. Dekant, K. Berthold, S. Schmidt, D. Wild, D. Henschler (1987). Enzymatic transformation of mercapturic acids derived from halogenated alkenes to reactive and mutagenic intermediates Biochemical Pharmacology, 36(17,17), 2741-2748
Data Type:	Preincubation assay - TCE
HERO ID:	65133

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name as TCE metabolite, N-Ac-DCVC , CASRN was not reported.
	Metric 2:	Test Substance Source	High	× 1	1	The compound was synthesized (methods provided), and analytically verified.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity >99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	Use of a concurrent negative control was not reported, nor were control results reported graphically.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Use of a concurrent positive control was not used or reported, but the results were reported to be positive.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were performed as described in another study with minimal additional details.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study design
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation of test solutions and storage were not reported.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Exposure methods were cited to another publication with no additional details
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations tested were not reported, but could be determined from data shown graphically
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration (120 min) was reported and appropriate for the study type.
	Metric 12:	Exposure Route and Method	High	× 1	1	3-4 exposure groups were tested for each assay condition. A dose-response was observed so the concentrations and spacing were appropriate for the outcome of interest.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Metabolic activation was reported (male Wistar rat kidney supernatant), and the concentration added was reported. Additional details on the source , isolation and other methodological details were not provided.
Domain 4: Test Model						

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Study Citation:	S. Vamvakas, W. Dekant, K. Berthold, S. Schmidt, D. Wild, D. Henschler (1987). Enzymatic transformation of mercapturic acids derived from halogenated alkenes to reactive and mutagenic intermediates Biochemical Pharmacology, 36(17,17), 2741-2748
Data Type:	Preincubation assay - TCE
HERO ID:	65133

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Low	× 2	6	S. typhimurium strain TA2638 was reported. This strain is not as commonly used as some others for mutagenicity assays. No additional details (including source) were reported.
	Metric 15:	Number per Group	Medium	× 1	2	Only a single strain was tested which is lower than the typical number used for this study type. The assays were performed in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Outcome assessment methodology was not described (assay cited to another publication).
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Outcome assessment was not described (assay cited to another publication).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial batch/lot number of organisms used per group was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical methods were not used. Even though studies were performed in triplicate, measures of variance were not provided.
	Metric 23:	Data Interpretation	Low	× 2	6	Scoring and evaluation criteria were not explicitly reported but text mentions doubling of spontaneous revertants which appears to be criterion for a positive result. Source of the number of spontaneous revertants was not reported but does not appear to be concurrent control.
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.
	Metric 25:	Reporting of Data	Medium	× 2	4	Data were reported graphically for the all treatment groups (means only; no measure of variability)
Overall Quality Determination‡			Unacceptable⋆⋆		2.2	
Extracted			No			

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Study Citation:	S. Vamvakas, W. Dekant, K. Berthold, S. Schmidt, D. Wild, D. Henschler (1987). Enzymatic transformation of mercapturic acids derived from halogenated alkenes to reactive and mutagenic intermediates Biochemical Pharmacology, 36(17,17), 2741-2748
Data Type:	Preincubation assay - TCE
HERO ID:	65133

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 95: **In vitro evaluation results for Furnus et al 1990 for Chinese hamster cell aneuploidy study**

Study Citation:	C. C. Furnus, M. A. Ulrich, M. C. Terreros, F. N. Dulout (1990). The induction of aneuploidy in cultured Chinese hamster cells by propionaldehyde and chloral hydrate Mutagenesis, 5(4,4), 323-326
Data Type:	Aneuploidy for chloral hydrate
HERO ID:	68820

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using a concurrent negative (untreated) control.
	Metric 5:	Positive Controls	High	× 2	2	Appropriate concurrent positive control test substances were included (acetaldehyde). Positive control groups exhibited positive responses.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were described and cited to previous publications (Dulout and Natarjan 1987; Dulout and Furnus 1988).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported (diluted in distilled water immediately before use). Storage was not reported (but was not likely to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups (3 plus controls) and dose spacing were appropriate. The high-dose was presumably based on data for the frequency of mitotic cells (data from pilot experiments).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 4: Test Model						
	Metric 14:	Test Model	Not Rated	NA	NA	The identity and passage number of the Chinese hamster embryonic diploid (CHED) cells were reported. Other information (origin and karyological characterization) was cited to other publications.

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Study Citation:	C. C. Furnus, M. A. Ulrich, M. C. Terreros, F. N. Dulout (1990). The induction of aneuploidy in cultured Chinese hamster cells by propionaldehyde and chloral hydrate Mutagenesis, 5(4,4), 323-326
Data Type:	Aneuploidy for chloral hydrate
HERO ID:	68820

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	Each experiment was repeated 5 times for chloral hydrate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	The sampling was somewhat lacking at 200 well-spread metaphases per experimental condition (approximately 300 typically used for studies of this type).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	The data were appropriately analyzed by Chi-squared test and variance analysis. However, statistical significance was mentioned only in the text (not shown in data tables); it was not always clear which CH data in the table were significantly different from controls.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (percentage of cells with aneuploidy) was reported and consistent with standards and guidelines.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity was not clearly defined. The study indicated that treatment lapses were selected to obtain similar mitotic indices, and that higher doses or longer treatment decreased the frequency of mitotic cells (in pilot experiments). However, the endpoint/methods of measurement were not clearly defined, and data were not shown.
	Metric 25:	Reporting of Data	High	× 2	2	All data are adequately reported.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

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Study Citation:	C. C. Furnus, M. A. Ulrich, M. C. Terreros, F. N. Dulout (1990). The induction of aneuploidy in cultured Chinese hamster cells by propionaldehyde and chloral hydrate Mutagenesis, 5(4,4), 323-326
Data Type:	Aneuploidy for chloral hydrate
HERO ID:	68820

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 96: **In vitro evaluation results for Furnus et al 1990 for Chinese hamster cell chromosomal aberration study**

Study Citation:	C. C. Furnus, M. A. Ulrich, M. C. Terreros, F. N. Dulout (1990). The induction of aneuploidy in cultured Chinese hamster cells by propionaldehyde and chloral hydrate Mutagenesis, 5(4,4), 323-326
Data Type:	Chromosomal aberration for chloral hydrate
HERO ID:	68820

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using a concurrent negative (untreated) control.
	Metric 5:	Positive Controls	High	× 2	2	Appropriate concurrent positive control test substances were included (acetaldehyde). Positive control groups exhibited positive responses.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were described and cited to previous publications (Dulout and Natarjan 1987; Dulout and Furnus 1988).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported (diluted in distilled water immediately before use). Storage was not reported (but was not likely to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (3 plus controls) and dose spacing were appropriate. The high-dose was presumably based on data for the frequency of mitotic cells (data from pilot experiments).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 4: Test Model						
	Metric 14:	Test Model	Not Rated	NA	NA	The identity and passage number of the Chinese hamster embryonic diploid (CHED) cells were reported. Other information (origin and karyological characterization was cited to other publications.

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Study Citation:	C. C. Furnus, M. A. Ulrich, M. C. Terreros, F. N. Dulout (1990). The induction of aneuploidy in cultured Chinese hamster cells by propionaldehyde and chloral hydrate Mutagenesis, 5(4,4), 323-326
Data Type:	Chromosomal aberration for chloral hydrate
HERO ID:	68820

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 15: Number per Group	High	× 1	1	Each experiment was repeated 5 times for chloral hydrate.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18: Sampling Adequacy	Medium	× 2	4	The sampling was somewhat lacking at 200 well-spread metaphases per experimental condition (approximately 300 typically used for studies of this type).
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were reported.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables were reported.
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	Medium	× 1	2	The data were appropriately analyzed by Chi-squared test and variance analysis. However, statistical significance was mentioned only in the text (not shown in data tables); it was not always clear which CH data in the table were significantly different from controls.
	Metric 23: Data Interpretation	High	× 2	2	Evaluation criteria (i.e., significantly increased frequency of chromosomal aberrations) was reported.
	Metric 24: Cytotoxicity Data	Low	× 1	3	Cytotoxicity was not clearly defined. The study indicated that treatment lapses were selected to obtain similar mitotic indices, and that higher doses or longer treatment decreased the frequency of mitotic cells (in pilot experiments). However, the endpoint/methods of measurement were not clearly defined, and data were not shown.
	Metric 25: Reporting of Data	High	× 2	2	All data are adequately reported.
Overall Quality Determination‡		High		1.4	
Extracted		Yes			

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Study Citation:	C. C. Furnus, M. A. Ulrich, M. C. Terreros, F. N. Dulout (1990). The induction of aneuploidy in cultured Chinese hamster cells by propionaldehyde and chloral hydrate Mutagenesis, 5(4,4), 323-326
Data Type:	Chromosomal aberration for chloral hydrate
HERO ID:	68820

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 97: **In vitro evaluation results for Costa and Ivanetich 1984 for rat hepatocyte unscheduled DNA synthesis study**

Study Citation:	A. K. Costa, K. M. Ivanetich (1984). Chlorinated ethylenes: their metabolism and effect on DNA repair in rat hepatocytes Carcinogenesis, 5(12,12), 1629-1636
Data Type:	UDS for TCE
HERO ID:	75075

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene (TCE).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (a manufacturer). Although a batch/lot number were not reported, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors report using a concurrent negative controls. DMSO was used as negative control substance (data shown); vehicle-only (ethanol) controls were also used (data not shown).
	Metric 5:	Positive Controls	High	× 2	2	Benzo[a]pyrene, a known carcinogen, was used as a positive control, and the intended positive response was induced.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were partially described and cited to Andrae and Schwarz (1981). Equipment used to measure absorbance was not reported.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported (dissolved in ethanol); storage was not reported (but was unlikely to affect the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were reported to be administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentration was reported without ambiguity (2.8 mM).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (2.5 hours) and considered appropriate for the study type (i.e., effective based on positive findings).

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Study Citation:	A. K. Costa, K. M. Ivanetich (1984). Chlorinated ethylenes: their metabolism and effect on DNA repair in rat hepatocytes Carcinogenesis, 5(12,12), 1629-1636
Data Type:	UDS for TCE
HERO ID:	75075

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	One concentration was used. This dose was justified by the study authors as "the highest concentration..tolerated by the hepatocytes." Since a positive result was observed, it is presumed that the test substance was tested at a dose sufficient to elicit a positive response without excessive cytotoxicity.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model (rat hepatocytes) was reported and is routinely used for the outcome of interest. The source of parent animals was not reported.
	Metric 15:	Number per Group	Medium	× 1	2	Experiments were reportedly repeated in as second set of experiments.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology appeared appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This method is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This method is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no confounding variables noted in the study. The study authors indicated that each experiment was conducted using hepatocytes from a single rat; viability of hepatocytes (>90%) was verified prior to use.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analysis is not required by study type (statistics were performed in the study, but not for this assay). Results (expressed in dpm and absorbance at 260 nm) were shown graphically.
	Metric 23:	Data Interpretation	Low	× 2	6	The study indicated that UDS was identified by a radioactive peak binding with parental DNA (coincident with the absorbance peak at 260 nm). Based on the data shown graphically, the determination/threshold for a positive result appears to be somewhat subjective.

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Study Citation:	A. K. Costa, K. M. Ivanetich (1984). Chlorinated ethylenes: their metabolism and effect on DNA repair in rat hepatocytes Carcinogenesis, 5(12,12), 1629-1636
Data Type:	UDS for TCE
HERO ID:	75075

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	The study indicated that the viability of cells was evaluated using the Trypan blue exclusion assay (without additional details). no data were shown.
	Metric 25:	Reporting of Data	Low	× 2	6	Data for the outcome was presented for the control and treatment group for one set of hepatocytes from a phenobarbital treated rat; a second set of experiments was noted to have identical results (+/- 5%, but was not reported). Data for the ethanol vehicle control were not shown, but reported to not stimulate UDS.
Overall Quality Determination‡			High		1.7	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 98: **In vitro evaluation results for Baden et al 1979 for bacterial reverse mutation study**

Study Citation:	J. M. Baden, M. Kelley, R. I. Mazze, V. F. Simmon (1979). Mutagenicity of inhalation anaesthetics: trichloroethylene, divinyl ether, nitrous oxide and cyclopropane British Journal of Anaesthesia, 51(5,5), 417-421
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	75270

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be over 99.5% pure, as measured by gas chromatography.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (room air).
	Metric 5:	Positive Controls	Medium	× 2	4	Positive controls (vinylidene chloride for dessicator incubation experiment, and 2-anthramine for liquid incubation experiment) were included in the experimental design, but it is unclear if they were tested concurrently with TCE or with another test substance. Positive controls yielded positive results.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were summarized and cited to another publication (Baden et al. 1976).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported. Test substance storage was not reported (this was not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The range of dose concentrations used (0.1 to 10%) was reported without ambiguity. Individual concentrations can be determined/estimated based on data in Figures 1 and 2.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration for both protocols was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (5 plus controls) and dose spacing were reported and appropriate.

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Study Citation:	J. M. Baden, M. Kelley, R. I. Mazze, V. F. Simmon (1979). Mutagenicity of inhalation anaesthetics: trichloroethylene, divinyl ether, nitrous oxide and cyclopropane British Journal of Anaesthesia, 51(5,5), 417-421
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	75270

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Medium	× 1	2	The source and method of preparation of the rat liver S9 fraction was reported; however, the concentration of S9 in the bacterial mutagenicity assay was not specified.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity and donor source of the bacterial strains used here were identified, and these strains are routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	Each assay was plated in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	Data were analyzed by Student's t-test (recommended as one of the ways to evaluate results for this study type). Fold-changes compared to controls were not described in the study report.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of colonies) was reported and consistent with current standards. The study used statistical significance as a criterion for positive results.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 25:	Reporting of Data	High	× 2	2	All data were adequately reported by exposure group. Data for Salmonella typhimurium strain TA 100 (dessicator and liquid incubation assays) were shown graphically in Figures 1 and 2. Data for S typhimurium strain TA 1535 were discussed qualitatively.

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Study Citation:	J. M. Baden, M. Kelley, R. I. Mazze, V. F. Simmon (1979). Mutagenicity of inhalation anaesthetics: trichloroethylene, divinyl ether, nitrous oxide and cyclopropane British Journal of Anaesthesia, 51(5,5), 417-421
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	75270

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		High		1.3	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 99: **In vitro evaluation results of Perocco and Prodi 1981 for unscheduled DNA synthesis**

Study Citation:	P. Perocco, G. Prodi (1981). DNA damage by haloalkanes in human lymphocytes cultured in vitro Cancer Letters, 13(3,3), 213-218
Data Type:	Unscheduled DNA synthesis (UDS) for TCE
HERO ID:	75278

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as trichloroethylene.
	Metric 2:	Test Substance Source	Medium	× 1	2	The sources of the test substances used in the study were identified (from Carlo Erba, Milan, Italy or Merck-Schuchardt), but it was unclear which test substances originated from which source.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of test substances used in the study ranged from 97-99% (purity of individual test substances not specified).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using concurrent negative controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were described adequately (e.g., cell density, volumes,temperature). The in vitro system used was partially cited to another publication.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The preparation of the test substance was reported; however, it was not explicitly indicated that microtest plates were covered (re: volatility of the test substance). Although the storage of the test substance was not reported, this omission is unlikely to impact the study results (single dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The final concentrations of the test substance used in the experiments was reported without ambiguity (in uL/mL).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (4 hr) was reported and appropriate for the outcome of interest.

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Study Citation: P. Perocco, G. Prodi (1981). DNA damage by haloalkanes in human lymphocytes cultured in vitro Cancer Letters, 13(3,3), 213-218
Data Type: Unscheduled DNA synthesis (UDS) for TCE
HERO ID: 75278

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of exposure groups was reported (3 treatment groups plus control). Results for two of the three treatment groups were obtained from a representative toxicity experiment; subsequent experiments used a single dose. The concentrations selected in the representative assay were not useful for evaluating a dose-response. The study indicates that the test substance induced toxicity at tested concentrations.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Rat liver phenobarbital-induced S9 mix was utilized. More detailed methods regarding metabolic activation were cited to other references.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	It was stated that healthy human volunteers were the origin of the blood samples from which the lymphocytes were isolated. However, no further information regarding gender, age, or other important demographics were included.
	Metric 15:	Number per Group	High	× 1	1	It was reported that six replicates were used per experimental condition.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	It was stated that healthy human volunteers were the origin of the blood samples from which the lymphocytes were isolated. However, it is unclear whether the 6 replicates for each experimental condition originated from 6 individual donors. It is also unclear whether different experimental conditions were tested on the same set of lymphocytes (e.g. Dose 1 tested on lymphocytes originated from donors A, B, and C; Dose 2 tested on lymphocytes originating from donors D, E, and F; etc).
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variables were reported.
Domain 7: Data Presentation and Analysis						

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Study Citation:	P. Perocco, G. Prodi (1981). DNA damage by haloalkanes in human lymphocytes cultured in vitro Cancer Letters, 13(3,3), 213-218
Data Type:	Unscheduled DNA synthesis (UDS) for TCE
HERO ID:	75278

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Unacceptable	× 1	4	Statistical analysis was not conducted and raw data were not provided, preventing an independent statistical analysis.
	Metric 23:	Data Interpretation	Low	× 2	6	The criteria for a positive response was not explicitly specified.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Scheduled DNA synthesis (SDS) was used as a measure of toxicity. Methods used to determine SDS were reported; however, cytotoxicity endpoints were not well-defined (i.e., the response that constituted a toxic effect).
	Metric 25:	Reporting of Data	Low	× 2	6	Data were reported by exposure group; however, data for experiments conducted with and without activation were not reported separately.
Overall Quality Determination‡			Unacceptable⋆⋆		1.9	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 100: **In vitro evaluation results for Dekant et al 1986 for bacterial reverse mutation study**

Study Citation:	W. Dekant, S. Vamvakas, K. Berthold, S. Schmidt, D. Wild, D. Henschler (1986). Bacterial beta-lyase mediated cleavage and mutagenicity of cysteine conjugates derived from the nephrocarcinogenic alkenes trichloroethylene, tetrachloroethylene and hexachlorobutadiene Chemico-Biological Interactions, 60(1,1), 31-45
Data Type:	Bacterial reverse mutation for DCVC
HERO ID:	75343

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	DCVC was identified by established nomenclature and chemical structure.
	Metric 2:	Test Substance Source	High	× 1	1	DCVC was synthesized and confirmed analytically by MS and NMR methods.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reportedly 98% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Solvent controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not used; however, this was a mechanistic paper designed to evaluate the metabolism and mutagenicity of cysteine conjugates of chlorinated alkenes. Typical positive controls used in Ames assays would not have been helpful in this study.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	The preincubation method was used;however, the method details were presented in a different publication (Maron and Ames, 1983).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric does not apply to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	DCVC was prepared in an organic solvent (not specified, but cited elsewhere) and tert-butoxycarbonyl-protecting group (boc) was added to increase the solubility of the cysteine-moiety in the organic solvent. No details on storage were provided.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Methodology details were presented in a different publication (Maron and Ames, 1983).
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported as nmol/plate.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	Methodology details were presented in a different publication (Maron and Ames, 1983).
	Metric 12:	Exposure Route and Method	High	× 1	1	5 concentrations were used and a dose-reponse relationship was observed.
	Metric 13:	Metabolic Activation	High	× 1	1	Metabolic activation is well described.
Domain 4: Test Model						

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Study Citation:	W. Dekant, S. Vamvakas, K. Berthold, S. Schmidt, D. Wild, D. Henschler (1986). Bacterial beta-lyase mediated cleavage and mutagenicity of cysteine conjugates derived from the nephrocarcinogenic alkenes trichloroethylene, tetrachloroethylene and hexachlorobutadiene Chemico-Biological Interactions, 60(1,1), 31-45
Data Type:	Bacterial reverse mutation for DCVC
HERO ID:	75343

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	The test model was routinely used for the outcome of interest. Strain properties were checked by testing the UV and crystal violet sensitivity, ampicillin resistance and mutability by UV light.
	Metric 15:	Number per Group	High	× 1	1	Multiple replicates were used (characterized as several) with consistent results.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment appered consistent across groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables were reported unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Dose-response curves were provided. Statistical analysis is not strictly required.
	Metric 23:	Data Interpretation	Low	× 2	6	Evaluation criteria were not strictly stated; however, conclusions were based on evaluation of dose-response curves.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity was described as increased formation of microcolonies (not further defined).
	Metric 25:	Reporting of Data	Medium	× 2	4	Data were not presented for results in TA98 (general result was summarized in text).
Overall Quality Determination‡			High		1.6	
Extracted			Yes			

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Study Citation:	W. Dekant, S. Vamvakas, K. Berthold, S. Schmidt, D. Wild, D. Henschler (1986). Bacterial beta-lyase mediated cleavage and mutagenicity of cysteine conjugates derived from the nephrocarcinogenic alkenes trichloroethylene, tetrachloroethylene and hexachlorobutadiene Chemico-Biological Interactions, 60(1,1), 31-45
Data Type:	Bacterial reverse mutation for DCVC
HERO ID:	75343

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 101: **In vitro evaluation results of Nestmann et al 1984 for Ames test study**

Study Citation:	E. R. Nestmann, R. Otson, D. J. Kowbel, P. D. Bothwell, T. R. Harrington (1984). Mutagenicity in a modified Salmonella assay of fabric-protecting products containing 1,1,1-trichloroethane Environmental and Molecular Mutagenesis, 6(1,1), 71-80
Data Type:	TCE Ames test
HERO ID:	194339

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name in the study.
	Metric 2:	Test Substance Source	High	× 1	1	The test substance was obtained from 2 sources: Fisher Scientific Co., Limited, and Aldrich Chemical Co. Lot numbers were provided as well.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and grade of test substance were not reported. However, GC and GC-MS analyses were described in detail.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A "no-dose" control (also referred to in the study as a "control (blank) chamber") was included in the study.
	Metric 5:	Positive Controls	Medium	× 2	4	Four positive controls were employed and results shown on data summary tables, though they were not discussed in the text.
	Metric 6:	Assay Procedures	Medium	× 1	2	Study authors cite methods described in Ames et al. (1975) and obtained the tester strains from the Ames lab. Study authors noted a test deviation (not incorporating test substances into the top agar but rather adding them to open Petri dishes in dessicators containing the culture dishes).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to this study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Study describes preparation and storage of gaseous standards of test substance and general preparation of liquid samples added to culture dishes, but does not discuss details of preparation or storage.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Nominal concentrations and time-weighted average exposure levels were reported for each exposure group.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	Incubation period was 24 hours exposure to test substance, followed by an additional 24 hours prior to scoring plates. The plate incorporation method requires a 48-72 hour exposure.

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Study Citation:	E. R. Nestmann, R. Otson, D. J. Kowbel, P. D. Bothwell, T. R. Harrington (1984). Mutagenicity in a modified Salmonella assay of fabric-protecting products containing 1,1,1-trichloroethane Environmental and Molecular Mutagenesis, 6(1,1), 71-80
Data Type:	TCE Ames test
HERO ID:	194339

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	Only 2 of 5 Salmonella strains were exposed to test substance.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Use of common metabolic activation system was reported, though not described in much detail.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Study employed commonly used bacterial strains and reported their source, but cited Ames et al. (1975) for a detailed description of them.
	Metric 15:	Number per Group	Low	× 1	3	Study employed 2 replicates/strain of bacteria. Initial bacterial cell counts were not reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology reported the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to mutagenicity assays
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable (no subjective outcomes were assessed)
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistical methods were not reported, though data tables noted general comparisons of plate counts to background.
	Metric 23:	Data Interpretation	High	× 2	2	Data were reported in such a way as to allow interpretation of test results.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Study did not evaluate cytotoxicity.
	Metric 25:	Reporting of Data	Low	× 2	6	Data were reported as revertants/plate for each exposure group, but data are insufficient to perform any statistical analysis (the only data reported is the mean of the duplicate plates).
Overall Quality Determination‡			Medium		1.9	
Extracted			Yes			

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Study Citation:	E. R. Nestmann, R. Otson, D. J. Kowbel, P. D. Bothwell, T. R. Harrington (1984). Mutagenicity in a modified Salmonella assay of fabric-protecting products containing 1,1,1-trichloroethane Environmental and Molecular Mutagenesis, 6(1,1), 71-80
Data Type:	TCE Ames test
HERO ID:	194339

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 102: **In vitro evaluation results of Amacher et al 1983 study on mammalian cell transformation**

Study Citation:	D. E. Amacher, I. Zelljadt (1983). The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to Salmonella typhimurium Carcinogenesis, 4(3,3), 291-296
Data Type:	Mammalian cell transformation for TCE
HERO ID:	194590

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (DMSO).
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were tested concurrently with each test substance. The identity of each positive control was reported (ethyl methanesulfonate and benzo[a]pyrene) and appropriate. Positive controls yielded positive results.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were adequately described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported. Test substance storage was not reported; however, solutions were prepared immediately before administration (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses are reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing was reported and appropriate for this assay.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to this study design.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity and method of isolation of the primary Syrian golden hamster embryo cells used here were reported and appropriate for the outcome of interest.

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Study Citation:	D. E. Amacher, I. Zelljadt (1983). The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to Salmonella typhimurium Carcinogenesis, 4(3,3), 291-296
Data Type:	Mammalian cell transformation for TCE
HERO ID:	194590

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The experiment was conducted with 30 wells per dose level per test substance.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to this study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each treatment group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	This metric is not applicable to the study design. Statistical analysis was not conducted on these data; any transformed colonies > 0 was considered a positive result. The raw data do not allow for an independent analysis because the data yielded from multiple doses per test substance were apparently pooled.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Scoring and evaluation criteria for assessing transformed colonies were cited to other publications.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	A preliminary toxicity assay was conducted to assess cytotoxicity levels. The doses for the mutagenicity assay were selected so that 50-90% survival was permitted. It is unclear what the methodology for assessing cytotoxicity was, and it is unclear whether cytotoxicity was assessed concurrently with the transformation assay.
	Metric 25:	Reporting of Data		× 2	NA	Raw data yielded from multiple dose levels per test substance were apparently pooled. Therefore, the data reporting is inadequate.
Overall Quality Determination‡			Unacceptable⋆⋆		1.5	
Extracted			No			

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Study Citation:	D. E. Amacher, I. Zelljadt (1983). The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to Salmonella typhimurium Carcinogenesis, 4(3,3), 291-296
Data Type:	Mammalian cell transformation for TCE
HERO ID:	194590

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 103: **In vitro evaluation results of Roldán-Arjona et al 1991 study on ara mutagenicity assay in S. typhimurium**

Study Citation:	T. Roldán-Arjona, M. D. García-Pedrajas, F. L. Luque-Romero, C. Hera, C. Pueyo (1991). An association between mutagenicity of the ara test of salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons Mutagenesis, 6(3,3), 199-205
Data Type:	ara mutagenicity assay in S. typhimurium- TCE
HERO ID:	194881

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as Trichloroethylene ("TCEL") with the correct CASRN and molecular formula.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (Aldrich). The product number and batch/lot number were not reported, but substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity and/or grade of the test substance was reported (provided by the supplier). 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors report using a solvent control (DMSO)
	Metric 5:	Positive Controls	Not Rated	NA	NA	A concurrent positive control was not used but may not be required for this study. The response of some known carcinogens tested in the study were positive and exhibited a dose-related response for mutations; this indicates that the assay was effective at inducing and identifying a positive mutagenic response.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were described; more detailed assay procedures were also described in a previously published studies (Hera and Pueyo, 1986; Roldan-Arjona et al., 1989)
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was described (dissolved in DMSO). Test substance storage was not reported, but this is appropriate given the study design (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were reported to be administered consistently across treated and control groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentration was reported in Table III without ambiguity

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Study Citation:	T. Roldán-Arjona, M. D. García-Pedrajas, F. L. Luque-Romero, C. Hera, C. Pueyo (1991). An association between mutagenicity of the ara test of salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons Mutagenesis, 6(3,3), 199-205
Data Type:	ara mutagenicity assay in S. typhimurium- TCE
HERO ID:	194881

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (20 minutes) and considered appropriate, as it yielded positive responses from a variety of chemicals tested and was in line with the Ames bacterial reverse mutation assay preincubation method exposure duration (also 20 minutes according to current standards).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number and spacing of exposure concentrations were reported in the results. It was noted that the investigator used a wide range of doses and the compound (negative for mutagenicity) gave a lethal response which indicated that bacteria were adequately exposed
	Metric 13:	Metabolic Activation	Medium	× 1	2	Assays were conducted with and without metabolic activation (S9 fraction from male rat liver induced with Aroclor-1254). The preparation of the S9 fraction was described in a previous publication (Maron and Ames, 1983). The source, concentration in the final culture and quality control information were not reported.
Domain 4: Test Model						
	Metric 14:	Test Model	Not Rated	NA	NA	The test model was reported along with limited descriptive information. The test model was routinely used for the outcome of interest. (S. typhimurium strains BA13 and BAL 13). The source of the bacteria strains were not specified in the report. These strains have been previously described in previously published reports (Ruiz-Rubio et al., 1985; Roldan-Arjona et al„ 1989)
	Metric 15:	Number per Group	Low	× 1	3	It was reported that at least two plates per dose level were used. This is not considered adequate by current standards for a similar assay (Ames bacterial reverse mutation requires 3 plates per dose level; use of 2 plates per dose level must be scientifically justified). Furthermore, the uncertainty regarding the number of plates per dose level ("at least two") indicates that the data yielded from each test substance and dose level were not obtained by identical procedures.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The AraR bacterial forward mutation assay appeared to be appropriate for the outcome of interest.

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Study Citation:	T. Roldán-Arjona, M. D. García-Pedrajas, F. L. Luque-Romero, C. Hera, C. Pueyo (1991). An association between mutagenicity of the ara test of salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons Mutagenesis, 6(3,3), 199-205
Data Type:	ara mutagenicity assay in S. typhimurium- TCE
HERO ID:	194881

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	The use of "at least two" plates per dose level indicates that the data yielded from each test substance and dose level were not obtained by identical procedures. It is not clear what the maximum amount of plates per dose level was, so the range of replicates used per dose level is unknown. This is considered to have potentially impacted results.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This method is not applicable to the outcome.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no confounding variables noted in the study
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variable unrelated to exposure were identified
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	A calculation for correlating number of mutations per unit time and per unit dose ("mutagenic potency") with previously established carcinogenic potency was given. However, statistical analysis was not conducted on the data. Although means and standard deviations are provided for each dose level, the number of plates per dose level is uncertain, and therefore independent statistical analysis cannot be conducted. However, statistical analysis is not necessarily required for the Ames bacterial reverse mutation assay, and due to the similarity of the AraR bacterial forward mutation assay, statistical analysis is considered to be not necessarily required for the present data.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria were reported and appropriate (test compound was considered mutagenic of the number of AraR mutant colonies was at least twice the value of the corresponding solvent control, over at least three dose levels)
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoints were described (survival)
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcome was presented for the control and treatment groups
Overall Quality Determination‡			High		1.3	

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Study Citation:	T. Roldán-Arjona, M. D. García-Pedrajas, F. L. Luque-Romero, C. Hera, C. Pueyo (1991). An association between mutagenicity of the ara test of salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons Mutagenesis, 6(3,3), 199-205
Data Type:	ara mutagenicity assay in S. typhimurium- TCE
HERO ID:	194881

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 104: **In vitro evaluation results for Milman et al 1988 for bacterial reverse mutation study**

Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	TCE bacterial reverse mutation
HERO ID:	200479

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by chemical name.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was reported as a range for multiple compounds (97-99% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	A concurrent negative control group was not included or reported.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A concurrent positive control or proficiency group was not used. A positive control is very commonly utilized in a bacterial reverse mutation assay. However, some test substances yielded positive responses, demonstrating that the assay was able to detect a positive response.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay procedures were cited to other publications (Ames et al., 1973a,b, 1975).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage was not reported.
	Metric 9:	Consistency of Exposure Administration	Unacceptable	× 1	4	Critical exposure details (e.g., amount of test substance used) were not reported.
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	The exposure doses/concentrations or amounts of test substance were not reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	No information on exposure duration(s) was reported, although assay procedure details were cited to other references.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups and dose/concentration spacing were not reported.
	Metric 13:	Metabolic Activation	Medium	× 1	2	A commonly used metabolic activation system was reported in the study; however, some details regarding type, composition mix, concentration, or quality control information were not described
Domain 4: Test Model						

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Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	TCE bacterial reverse mutation
HERO ID:	200479

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 14: Test Model	Low	× 2	6	The test model was reported but no additional details were given.
	Metric 15: Number per Group	Unacceptable	× 1	4	Replicates per study group were not reported.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported and sensitive for the outcome of interest.
	Metric 17: Consistency of Outcome Assessment	Low	× 1	3	Details were not reported regarding the execution of the study protocol for outcome assessment.
	Metric 18: Sampling Adequacy	Not Rated	NA	NA	Not applicable to the outcome of interest.
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	Not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions per study group were not reported.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	Not Rated	NA	NA	No quantitative data were provided.
	Metric 23: Data Interpretation	Medium	× 2	4	Evaluation criteria were partially reported.
	Metric 24: Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.
	Metric 25: Reporting of Data	Low	× 2	6	Text indicated that TCE was "reproducibly mutagenic to base-pair substitution tester strains TA1535 and TA100 in the presence and absence of the metabolic activation systems". No quantitative data was reported.
Overall Quality Determination‡		Unacceptable⋆⋆		3.2	
Extracted		No			

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Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	TCE bacterial reverse mutation
HERO ID:	200479

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 105: **In vitro evaluation results for Milman et al 1988 for hepatocyte DNA repair study**

Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	TCE hepatocyte DNA repair
HERO ID:	200479

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by chemical name.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was reported as a range for multiple compounds (97-99% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	A concurrent negative control group was not included or reported.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric may not be applicable to the DNA repair test.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay procedures were cited to other publications (Williams 1976, 1977).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage was not reported.
	Metric 9:	Consistency of Exposure Administration	Unacceptable	× 1	4	Critical exposure details (e.g., amount of test substance used) were not reported.
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	The exposure doses/concentrations or amounts of test substance were not reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	No information on exposure duration(s) was reported, although assay procedure details were cited to other references.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups and dose/concentration spacing were not reported.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not needed for primary hepatocytes.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was reported but no additional details were given.
	Metric 15:	Number per Group	High	× 1	1	Triplicates were indicated.
Domain 5: Outcome Assessment						

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Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	TCE hepatocyte DNA repair
HERO ID:	200479

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Details were not reported regarding the execution of the study protocol for outcome assessment.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions per study group were not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	No quantitative data were provided.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were partially reported.
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.
	Metric 25:	Reporting of Data	Low	× 2	6	Text indicated that TCE "elicited a positive response with hepatocytes from male B6C3F1 mice only". No quantitative data was provided.
Overall Quality Determination‡			Unacceptable⋆⋆		3.0	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 106: **In vitro evaluation results for Milman et al 1988 for cell transformation study**

Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	TCE cell transformation
HERO ID:	200479

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by chemical name.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was reported as a range for multiple compounds (97-99% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	A negative control was referenced briefly in the results, but no details were provided and results were not reported for negative controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric may not be applicable to the cell transformation assay.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay procedures were cited to other publications (Sivak and Tu, 1980).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage was not reported.
	Metric 9:	Consistency of Exposure Administration	Unacceptable	× 1	4	Critical exposure details (e.g., amount of test substance used) were not reported.
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	Concentrations were reproted as a range (20-250 ug/mL), but it is not clear whether these were the only concentrations tested.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	No information on exposure duration(s) was reported, although assay procedure details were cited to other references.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups and dose/concentration spacing were not reported.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not needed.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was reported but no additional details were given.
	Metric 15:	Number per Group	Not Rated	NA	NA	Not indicated; possibly cited to another publication (Sivak and Tu, 1980)

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Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	TCE cell transformation
HERO ID:	200479

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Details were not reported regarding the execution of the study protocol for outcome assessment.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions per study group were not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	No quantitative data were provided.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were partially reported.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity was assessed; however, methods were not described.
	Metric 25:	Reporting of Data	Low	× 2	6	Text indicated that TCE was "marginally positive" with a dose-dependent increase, although the increase was not statistically significant. Quantitative data were not reported.
Overall Quality Determination‡			Unacceptable⋆⋆		2.8	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 107: **In vitro evaluation results for Vamvakas et al 1988 for micronucleus study**

Study Citation:	S. Vamvakas, W. Dekant, D. Schiffmann, D. Henschler (1988). Induction of unscheduled DNA synthesis and micronucleus formation in Syrian hamster embryo fibroblasts treated with cysteine S-conjugates of chlorinated hydrocarbons Cell Biology and Toxicology, 4(4,4), 393-403
Data Type:	MN for DCVC
HERO ID:	200648

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as S-(1,2-dichlorovinyl)-cysteine (DCVC), a metabolite of TCE.
	Metric 2:	Test Substance Source	High	× 1	1	The test substance was synthesized and verified by TLC and/or HPLC analysis.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was at least 99% (based on TLC and/or HPLC analysis).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study authors reported using a negative (solvent-only) control. However, the results section provided data for "untreated monolayers;" it's unclear if these data refer to solvent controls.
	Metric 5:	Positive Controls	High	× 2	2	4-Nitroquinoline-1-oxide (1-NQO) was used as a positive control. Although positive controls were reportedly used, the results provided a value for 4-NQO after 18 hours (but not 30 hours) incubation; it's unclear if this value (155+/-13.2) is representative of multiple assays.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were described briefly and cited to Schmuck et al. (1988).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was presumably dissolved in methanol (explicitly specified for the UDS assay but not the MN assay). Storage was not reported (but not likely to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures appeared to be administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The molar concentrations used in the UDS assay were presumably used for the MN assay (but this is not explicitly specified).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (1 to 10 hours treatment with 6 to 36 hours incubation).

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Study Citation: S. Vamvakas, W. Dekant, D. Schiffmann, D. Henschler (1988). Induction of unscheduled DNA synthesis and micronucleus formation in Syrian hamster embryo fibroblasts treated with cysteine S-conjugates of chlorinated hydrocarbons Cell Biology and Toxicology, 4(4,4), 393-403
Data Type: MN for DCVC
HERO ID: 200648

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of exposure groups was reported for the UDS assay (5 plus controls); based on data for another substance tested in the study, the same or some of these concentrations were used for the MN assay. The study authors indicated that highest concentration selected for testing was the lowest cytotoxic dose.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required for the study; the metabolite was directly tested.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model (Syrian hamster embryo [SHE] fibroblasts) was with limited descriptive information (tertiary cultures from 13-14-day old embryos, established in the laboratory- not from a commercial source). The test model was routinely used for genotoxicity tests. The laboratory aimed to use the cell type for multiple assays to evaluate the mechanism of genotoxic action.
	Metric 15:	Number per Group	High	× 1	1	All experiments were repeated twice, and every concentration was tested twice within each experiment.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate. The duration of exposure and incubation was optimized to ensure that the assay was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Exposures were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The study indicated that 2000 cells per concentration were scored.
	Metric 19:	Blinding of Assessors	High	× 1	1	Slides were randomized prior to scoring to prevent knowledge of treatment protocol.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on confounding variables not related to exposure were not reported.
Domain 7: Data Presentation and Analysis						

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Study Citation:	S. Vamvakas, W. Dekant, D. Schiffmann, D. Henschler (1988). Induction of unscheduled DNA synthesis and micronucleus formation in Syrian hamster embryo fibroblasts treated with cysteine S-conjugates of chlorinated hydrocarbons Cell Biology and Toxicology, 4(4,4), 393-403
Data Type:	MN for DCVC
HERO ID:	200648

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Unacceptable	× 1	4	It does not appear that statistical analyses were performed. No quantitative data for independent analyses were provided.
	Metric 23:	Data Interpretation	Low	× 2	6	The criteria for a positive response (in the absence of statistical analyses) was not specified. The study indicated only that MNs for DCVC were not "above control rates." Results for another chemical used in the study described the dose- and time-relatedness of the response.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity testing was reported (LDH release measurements briefly described) and was used to determine doses. The study indicated that cytotoxicity was monitored in all UDS experiments (and presumably applicable to MN experiment too). The cytotoxicity data provided were different doses than used in the genotoxicity assays.
	Metric 25:	Reporting of Data	Low	× 2	6	Negative results were reported qualitatively.
Overall Quality Determination‡			Unacceptable⋆⋆		1.8	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 108: **In vitro evaluation results for Vamvakas et al 1988 for unscheduled DNA synthesis study**

Study Citation:	S. Vamvakas, W. Dekant, D. Schiffmann, D. Henschler (1988). Induction of unscheduled DNA synthesis and micronucleus formation in Syrian hamster embryo fibroblasts treated with cysteine S-conjugates of chlorinated hydrocarbons Cell Biology and Toxicology, 4(4,4), 393-403
Data Type:	UDS for DCVC
HERO ID:	200648

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as S-(1,2-dichlorovinyl)-cysteine (DCVC), a metabolite of TCE.
	Metric 2:	Test Substance Source	High	× 1	1	The test substance was synthesized and verified by TLC and/or HPLC analysis.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was at least 99% (based on TLC and/or HPLC analysis).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study authors reported using concurrent negative (solvent-only and/or untreated) controls. It was not clear if the two terms were used interchangeably, or if both types of controls were used.
	Metric 5:	Positive Controls	Medium	× 2	4	4-Nitroquinoline-1-oxide (1-NQO) was used as a positive control. Although positive controls were reportedly used in every experiment, the results provided a value for 4-NQO after 10 hours exposure; it's unclear if this value (35,830 +/-2620 dpm/culture) is representative of multiple assays (the main UDS assay for DCVC was 24 hours in duration).
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were described as a modification of the protocol described by Schiffmann et al. (1984).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	It was indicated that the test substance was dissolved in methanol. Storage was not reported (but not likely to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures appeared to be administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Molar concentrations of DCVC were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The duration of exposures was clearly reported (and optimized); based on results, the duration appeared appropriate for the study type.

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Study Citation:	S. Vamvakas, W. Dekant, D. Schiffmann, D. Henschler (1988). Induction of unscheduled DNA synthesis and micronucleus formation in Syrian hamster embryo fibroblasts treated with cysteine S-conjugates of chlorinated hydrocarbons Cell Biology and Toxicology, 4(4,4), 393-403
Data Type:	UDS for DCVC
HERO ID:	200648

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups was reported (5 plus controls). The study authors indicated that highest concentration selected for testing was the lowest cytotoxic dose.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required for the study; the metabolite was directly tested.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model (Syrian hamster embryo [SHE] fibroblasts) was with limited descriptive information (tertiary cultures from 13-14-day old embryos, established in the laboratory- not from a commercial source). The test model was routinely used for genotoxicity tests. The laboratory aimed to use the cell type for multiple assays to evaluate the mechanism of genotoxic action.
	Metric 15:	Number per Group	High	× 1	1	The study indicated that measurements were made in triplicate, and all experiments were repeated at least twice.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate. The duration of exposure was optimized to ensure that the assay was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Exposures were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on confounding variables not related to exposure were not reported.
Domain 7: Data Presentation and Analysis						

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Study Citation:	S. Vamvakas, W. Dekant, D. Schiffmann, D. Henschler (1988). Induction of unscheduled DNA synthesis and micronucleus formation in Syrian hamster embryo fibroblasts treated with cysteine S-conjugates of chlorinated hydrocarbons Cell Biology and Toxicology, 4(4,4), 393-403
Data Type:	UDS for DCVC
HERO ID:	200648

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analysis was presumably not conducted. Data were provided (i.e., means plus SD for n =6 presented graphically for the exposure groups with the exception of controls). The study stated that background levels of 3H-thymidine incorporation (from residual replicative synthesis) in untreated cells or in the presence of methanol were subtracted; the negative control value of 5139+/-850 dpm/culture well (for 24 hours) was given; it's unclear if this value was derived from multiple assays.
	Metric 23:	Data Interpretation	Low	× 2	6	Evaluation criteria was not adequately described. Based on information in the results, the dose-relatedness of the response was considered. The response for DCVC was described as "less pronounced but clearly dose-dependent," but the threshold for a positive response was not specified.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity testing was reported (LDH release measurements briefly described) and was used to determine doses for the UDS assay. Although the study indicated that cytotoxicity was monitored in all UDS experiments, the cytotoxicity data provided were different doses than used in the UDS study.
	Metric 25:	Reporting of Data	Low	× 2	6	Data for UDS was presented graphically for all exposure groups excluding controls); tabular UDS data were not provided.
Overall Quality Determination‡			Medium		1.9	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 109: **In vitro evaluation results of Hasspieler et al 2006 for DNA SSBs and repair**

Study Citation:	Hasspieler, B., Haffner, D., Stelljes, M., Adeli, K. (2006). Toxicological assessment of industrial solvents using human cell bioassays: assessment of short-term cytotoxicity and long-term genotoxicity potential Toxicology and Industrial Health, 22(7,7), 301-315
Data Type:	DNA SSBs and repair for TCE
HERO ID:	478653

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name, CASRN, and structural formula.
	Metric 2:	Test Substance Source	High	× 1	1	The test substance source (a manufacturer) was reported. Although a batch/lot number were not reported, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	The test substance purity/grade was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using negative (solvent-only) controls. The study indicated that DMSO and acetone were used; however, the solvent used for TCE was not explicitly specified.
	Metric 5:	Positive Controls	High	× 2	2	The study authors reported using a positive control for the DNA damage and repair assays (4-nitroquinoline N-oxide).
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods/procedures were described, but specific details were not reported (e.g., volumes). It was indicated that the procedure used for analyzing DNA SSB assay was a modification of a procedure cited to another publication (Hasspieler et al. 1995).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	It was indicated that the test substance was dissolved in solvent. Storage was not reported (but it not expected to impact the study results given the short-term nature of the experiments).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration appeared to be consistent across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	A range of doses tested was reported (25 to 500 ppm). Individual doses can be estimated from data presented in Figure 3.

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Study Citation:	Hasspieler, B., Haffner, D., Stelljes, M., Adeli, K. (2006). Toxicological assessment of industrial solvents using human cell bioassays: assessment of short-term cytotoxicity and long-term genotoxicity potential Toxicology and Industrial Health, 22(7,7), 301-315
Data Type:	DNA SSBs and repair for TCE
HERO ID:	478653

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	The exposure duration for other assays performed in the study were up to 24 hours (cytotoxicity) or 24 hours (EROD bioassay). Descriptions of the genotoxicity assays (DNA SSB and repair assays) reported treatments "for a given period of time," and reference information described above for other assay types. The duration of exposure for the genotoxicity assays was not explicitly specified (DNA SSB duration may be included in a cited publication and/or 24 hours may be presumed). Based on positive results (e.g., for the positive control), the exposure duration was presumably adequate for the outcome of interest.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of exposure groups was reported (i.e., can be determined based on the data presented in Figure 3). A rationale for dose selection was suggested (similar to expected tissue concentrations); however, all doses used for TCE caused substantial toxicity (survival < 25% relative to controls).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model (human HepG2 cells) was reported and is routinely used for toxicity studies. The source of the cell line was specified, but few details were provided.
	Metric 15:	Number per Group	High	× 1	1	The legend for Figure 3 indicates that four replicates were used.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methods were described and appeared appropriate for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessments appeared to be consistent across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Test design or procedural confounding variables were not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables in health outcomes unrelated to exposure were reported.

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Study Citation:	Hasspieler, B., Haffner, D., Stelljes, M., Adeli, K. (2006). Toxicological assessment of industrial solvents using human cell bioassays: assessment of short-term cytotoxicity and long-term genotoxicity potential Toxicology and Industrial Health, 22(7,7), 301-315
Data Type:	DNA SSBs and repair for TCE
HERO ID:	478653

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	Medium	× 1	2	Data were shown in Figure 3 as means +/- standard error for 4 replicates (this statement presumably pertains to all of the assays). It was indicated that statistical analyses were performed (threshold $p < 0.05$); however, details of tests conducted were not provided.
	Metric 23: Data Interpretation	High	× 2	2	Based on information provided in Table 2, a test was scored as positive when percent change in activity was statistically significantly different from the negative control.
	Metric 24: Cytotoxicity Data	High	× 1	1	Cytotoxicity methods were described; these methods (neutral red uptake assay) are commonly used.
	Metric 25: Reporting of Data	High	× 2	2	Data for all exposure groups were presented graphically (Figure 3). The data were summarized in Table 2.
Overall Quality Determination‡		High		1.6	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 110: **In vitro evaluation results for Emmert et al 2006 for bacterial reverse mutation study**

Study Citation:	B. Emmert, J. Bünger, K. Keuch, M. Müller, S. Emmert, E. Hallier, G. A. Westphal (2006). Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S. typhimurium strain YG7108pin3ERb5 Toxicology, 228(1,1), 66-76
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	597695

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as trichloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported. An analysis number was also provided.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be at least 99.5% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study indicated that the test substance was tested as a solution in DMSO. However, the legend for Figure 8 states microcolony induction by the test substance (10 to 25 ug/uL in ethanol). There is uncertainty as to the vehicle-control substance that was used.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls (N-nitrosodiethylamine) were included in the experimental design. Positive controls yielded positive results.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were described and partially cited to another publication. The study indicated that the Ames test was carried out according to Maron and Ames (1983) with slight modifications owing to the bacterial strain that was used in the study.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported. Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (i.e., could be estimated from data shown in Figure 8).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate. The authors provided a justification for an extended exposure time (i.e., the strain grows slowly in the presence of toxicants).

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Study Citation:	B. Emmert, J. Bünger, K. Keuch, M. Müller, S. Emmert, E. Hallier, G. A. Westphal (2006). Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S. typhimurium strain YG7108pin3ERb5 Toxicology, 228(1,1), 66-76
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	597695

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups was reported (can be ascertained based on data shown in Figure 8). The study indicated that test substances were initially tested up to 5 mg/plate, toxic concentrations, or the highest soluble concentration (to determine the concentration range for the mutagenicity assay).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type. Conventional S9 activation was used for some assays (but not for this test substance). The bacterial strain used in this assay conferred metabolic competence (including CYP P450 2E1).
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was provided with some descriptive information. The strain appeared to be laboratory-maintained;the strain had to be transformed with a plasmid for each test (because large plasmids are often lost). The strain has not been routinely used in studies of this type.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was conducted 5 times.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology (numbers of revertant colonies) is routinely used for the outcome of interest. However, the sensitivity of the assay to detect an effect is uncertain (the authors indicated that cyototoxic metabolites were produced by the metabolically-competent bacterial strain used in the assay). The study states that either the metabolites generated by the strain were not mutagenic, the strain is not sensitive for these compounds, or the bacteria masks possible mutagenic effects.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type. Colony counting was conducted automatically.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were reported.

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Study Citation:	B. Emmert, J. Bünger, K. Keuch, M. Müller, S. Emmert, E. Hallier, G. A. Westphal (2006). Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S. typhimurium strain YG7108pin3ERb5 Toxicology, 228(1,1), 66-76
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	597695

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	The study does not indicate that statistical analysis was conducted; this analysis is not required by study type (fold-changes relative to control are evaluated). Data were presented as means +/- standard deviations.
	Metric 23:	Data Interpretation	High	× 2	2	The study clearly specified the criteria for a positive result. Results were considered positive if at least 2 consecutive doses were 2x baseline with dose-dependency.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Not required by study type. The study eluded to preliminary toxicity testing to define the dose range (not further described).
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group for microcolony induction (indicative of toxicity). Data for mutagenicity were qualitative (indicated as negative).
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 111: **In vitro evaluation results for von der Hude et al 1988 for bacterial mutagenicity study**

Study Citation:	W. von der Hude, C. Behm, R. Gürtler, A. Basler (1988). Evaluation of the SOS chromotest Mutation Research, 203(2,2), 81-94					
Data Type:	TCE SOS chromotest in E coli PQ37					
HERO ID:	627708					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	test substance reported by name and CAS
	Metric 2:	Test Substance Source	Low	× 1	3	test substance source was not reported
	Metric 3:	Test Substance Purity	Low	× 1	3	test substance purity was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	concurrent negative (solvent) control was reported
	Metric 5:	Positive Controls	High	× 2	2	concurrent positive controls were included in the presence (BaP) and absence (4-NQO) of metabolic activation
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were previously cited, and briefly reported and appropriate for the study
	Metric 7:	Standards for Tests	Not Rated	NA	NA	not applicable for the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	test substance storage was not reported but is unlikely to impact this short duration study. Preparation was inferred (dissolved in solvent)
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	exposure methods were briefly described and cited to previous publication
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	Concentrations were not specified; reported in methods as 3-5 concentrations at half log intervals up to the limit of solubility or 100 mM
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	exposure duration was 2h incubation period and was adequate for the study type
	Metric 12:	Exposure Route and Method	High	× 1	1	number of exposure groups was consistent with standards (3-5) and spacing was based on solubility
	Metric 13:	Metabolic Activation	Medium	× 1	2	metabolic activation was reported, commonly used, and details were cited to other publications
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Test model (E. coli PQ37) was reported with limited descriptive information. It is routinely used for the outcome of interest. The test model was not obtained from a commercial source but a private individual.

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Study Citation: W. von der Hude, C. Behm, R. Gürtler, A. Basler (1988). Evaluation of the SOS chromotest Mutation Research, 203(2,2), 81-94
Data Type: TCE SOS chromotest in E coli PQ37
HERO ID: 627708

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	Optical density of experimental cultures was reported and consistent across groups. Study reports validation of results in independent assays (n not reported)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	outcome assessment methodology (SOS chromotest) was described and appeared appropriate for the study
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	outcome assessment was carried out consistently across groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	not applicable for the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	not applicable for the study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	statistical analysis was not described but is not necessary for this outcome
	Metric 23:	Data Interpretation	High	× 2	2	evaluation criteria were reported and consistent with standard practice
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Alkaline phosphatase portion of assay is a measure of cytotoxicity; however, results were not reported for test chemical
	Metric 25:	Reporting of Data	Low	× 2	6	Results were reported qualitatively and in summary form in Table 3
Overall Quality Determination‡			Unacceptable**		1.8	
Extracted			No			

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 112: **In vitro evaluation results for Degrassi and Tanzarella 1988 for micronucleus and chromosomal aberration study**

Study Citation:	F. Degrassi, C. Tanzarella (1988). Immunofluorescent staining of kinetochores in micronuclei: A new assay for the detection of aneuploidy Mutation Research, 203(5,5), 339-345
Data Type:	Micronucleus and chromosome aberration assays
HERO ID:	628744

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chloral hydrate (CH) was identified by established nomenclature.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was reported. Batch/lot number was not reported; however, the test substance composition is not expected to vary.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity/grade was not reported; however, because the test substance was obtained from a commercial source, this is not expected to have substantially impacted results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Study authors acknowledged using a concurrent negative control group, but details regarding the negative control group were not reported. It may have been an untreated control, as it served as a control for x-ray treatments as well.
	Metric 5:	Positive Controls	High	× 2	2	X-rays and colchicine may be considered as positive controls for chloral hydrate.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods were well reported.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable for the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage was not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was described and inferred to be consistent across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate for the study type.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Concentrations were not justified, but were adequate to evaluate a timecourse of exposure (15-48 hr).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not described; but it is not clear whether it would be necessary for hamster C1-1 cells.
Domain 4: Test Model						

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Study Citation: F. Degrassi, C. Tanzarella (1988). Immunofluorescent staining of kinetochores in micronuclei: A new assay for the detection of aneuploidy Mutation Research, 203(5,5), 339-345
Data Type: Micronucleus and chromosome aberration assays
HERO ID: 628744

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The academic source of the cell line was reported and cellular properties were described.
	Metric 15:	Number per Group	High	× 1	1	Results were reproduced in 3 separate experiments (3 replicates per treatment group).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methods were reported and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment details were reported and appeared consistent across groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	Micronuclei in 1000 cells and chromosome aberrations in 100 metaphases. These sampling sizes were specified to be for each experimental point (replicate), so the sampling size was adequate according to current standards and guidelines.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences were reported among study group parameters.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variables unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Unacceptable	× 1	4	Statistics were reported and appropriate for CA. Statistics were not described for the micronuclei data and could not be analyzed independently because no standard deviation was given; therefore, this is considered to be unacceptable.
	Metric 23:	Data Interpretation	Low	× 2	6	Evaluation criteria were not reported.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity was described as "a strong C-mitotic effect", but the methods of measuring this effect were not described.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.
Overall Quality Determination‡			Unacceptable⋆⋆		1.5	
Extracted			No			

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Study Citation:	F. Degrassi, C. Tanzarella (1988). Immunofluorescent staining of kinetochores in micronuclei: A new assay for the detection of aneuploidy Mutation Research, 203(5,5), 339-345
Data Type:	Micronucleus and chromosome aberration assays
HERO ID:	628744

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 113: **In vitro evaluation results for Demarini et al 1994 for bacterial reverse mutation study**

Study Citation:	D. M. Demarini, E. Perry, M. L. Shelton (1994). Dichloroacetic acid and related compounds: Induction of prophage in E. coli and mutagenicity and mutation spectra in Salmonella TA100 Mutagenesis, 9(5,5), 429-437
Data Type:	Reverse mutation for TCE and metabolites (DCA, DCAC, TCOH)
HERO ID:	628757

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	test substances were reported by name, CASRN, and molecular weight
	Metric 2:	Test Substance Source	High	× 1	1	test substance source (Sigma) was reported, batch/lot was not reported but composition is not expected to vary
	Metric 3:	Test Substance Purity	High	× 1	1	Purity of all chemicals was reported to be 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	concurrent negative controls were used, but it is unclear if they were untreated or vehicle controls.
	Metric 5:	Positive Controls	High	× 2	2	concurrent positive controls (sodium azide without S9 and 2-AA with S9) were used with and without metabolic activation
	Metric 6:	Assay Procedures	Medium	× 1	2	assay procedures were cited to a prior publication, and modifications were described and appeared appropriate
	Metric 7:	Standards for Tests	Not Rated	NA	NA	not applicable for the study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was accomplished by injection into the sealed bag . Storage was not reported but is unlikely to impact this short term study.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposure methods were cited to a prior publication and briefly described and appeared to be consistent across groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	concentrations were reported in figure 3 (in mg/ml) and can be estimated/quantified
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	exposure duration was 24h and appears to be adequate for the study
	Metric 12:	Exposure Route and Method	High	× 1	1	concentrations (4 plus control) and spacing were reported; high concentration justified by authors as up to cytotoxic doses
	Metric 13:	Metabolic Activation	Medium	× 1	2	metabolic activation was reported and commonly used; preparation was cited to another publication
Domain 4: Test Model						

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Study Citation:	D. M. Demarini, E. Perry, M. L. Shelton (1994). Dichloroacetic acid and related compounds: Induction of prophage in E. coli and mutagenicity and mutation spectra in Salmonella TA100 Mutagenesis, 9(5,5), 429-437
Data Type:	Reverse mutation for TCE and metabolites (DCA, DCAC, TCOH)
HERO ID:	628757

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	Test model (S typhimurium strain TA100) was briefly characterized and is appropriate for the study type. Test model was not obtained from commercial source but from private researcher. Specific single strain was selected with justification for evaluation of specific revertant codon mutation
	Metric 15:	Number per Group	Medium	× 1	2	Each experiment performed at least twice
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology (colony counting) was reported (Automatic colony counter) and appropriate
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Consistent outcome assessment across groups is inferred from the text
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	not applicable for the study
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	not applicable for the study
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	statistical analysis was not performed but is not required for this study type
	Metric 23:	Data Interpretation	High	× 2	2	Criterion for a positive response was a reproducible 2-fold increase in revertants/plate over background and is consistent with standard practice
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were defined, but the methods of measurements were not fully described or reported
	Metric 25:	Reporting of Data	High	× 2	2	Results reported for each concentration and each experiment as a mean and SEM of duplicate plates
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

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Study Citation:	D. M. Demarini, E. Perry, M. L. Shelton (1994). Dichloroacetic acid and related compounds: Induction of prophage in E. coli and mutagenicity and mutation spectra in Salmonella TA100 Mutagenesis, 9(5,5), 429-437
Data Type:	Reverse mutation for TCE and metabolites (DCA, DCAC, TCOH)
HERO ID:	628757

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 114: **In vitro evaluation results for Demarini et al 1994 for bacterial DNA damage study**

Study Citation:	D. M. Demarini, E. Perry, M. L. Shelton (1994). Dichloroacetic acid and related compounds: Induction of prophage in E. coli and mutagenicity and mutation spectra in Salmonella TA100 Mutagenesis, 9(5,5), 429-437
Data Type:	DNA damage (prophage induction) for TCE and metabolites (DCA, DCAC, TCOH)
HERO ID:	628757

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	test substances were reported by name, CASRN, and molecular weight
	Metric 2:	Test Substance Source	High	× 1	1	test substance source (Sigma) was reported, batch/lot was not reported but composition is not expected to vary
	Metric 3:	Test Substance Purity	High	× 1	1	Purity of all chemicals was reported to be 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	concurrent negative controls (media) were reported
	Metric 5:	Positive Controls	High	× 2	2	concurrent positive controls (2-nitrofluorene without S9 and 2-aminoanthracine with S9) were used
	Metric 6:	Assay Procedures	High	× 1	1	assay procedures were cited to a prior publication, briefly described and appeared appropriate for the study type
	Metric 7:	Standards for Tests	Not Rated	NA	NA	not applicable for the study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was a dilution series in medium. Storage was not reported but is unlikely to impact this short term study.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposure methods were cited to a prior publication and briefly described and appeared to be consistent across groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	concentrations were reported in figure 2 (in mg/ml) and can be estimated/quantified
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	exposure duration was an overnight incubation, not further described but appeared to be appropriate for the study type
	Metric 12:	Exposure Route and Method	High	× 1	1	concentrations (4 plus control) and spacing were reported; high concentration justified by authors as up to cytotoxic doses
	Metric 13:	Metabolic Activation	High	× 1	1	metabolic activation was reported and commonly used; preparation was cited to another publication
Domain 4: Test Model						

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Study Citation:	D. M. Demarini, E. Perry, M. L. Shelton (1994). Dichloroacetic acid and related compounds: Induction of prophage in E. coli and mutagenicity and mutation spectra in Salmonella TA100 Mutagenesis, 9(5,5), 429-437
Data Type:	DNA damage (prophage induction) for TCE and metabolites (DCA, DCAC, TCOH)
HERO ID:	628757

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	Test model (E coli) was briefly characterized and is appropriate for the study type. Test model was not obtained from commercial source but from private researcher.
	Metric 15:	Number per Group	Medium	× 1	2	Each experiment performed at least twice
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	outcome assessment methodology (hand counting of plaque forming units) was described and appeared appropriate for the outcome of interest
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Consistent outcome assessment across groups is inferred from the text
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	not applicable for the study
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	not applicable for the study
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	statistical analysis was not performed but is not required for this study type
	Metric 23:	Data Interpretation	High	× 2	2	Criterion for a positive response was 3-fold increase in PFU/plate over background and reproducible dose dependent increase and is consistent with standards and previous citations
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were defined, but the methods of measurements were not fully described or reported
	Metric 25:	Reporting of Data	High	× 2	2	Results reported for each concentration and each experiment as a mean and SEM of duplicate plates
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

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Study Citation:	D. M. Demarini, E. Perry, M. L. Shelton (1994). Dichloroacetic acid and related compounds: Induction of prophage in E. coli and mutagenicity and mutation spectra in Salmonella TA100 Mutagenesis, 9(5,5), 429-437
Data Type:	DNA damage (prophage induction) for TCE and metabolites (DCA, DCAC, TCOH)
HERO ID:	628757

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 115: **In vitro evaluation results for Kafer 1986 for fungal chromosomal aberration study**

Study Citation:	E. Käfer (1986). Tests which distinguish induced crossing-over and aneuploidy from secondary segregation in Aspergillus treated with chloral hydrate or gamma-rays Mutation Research, 164(3,3), 145-166
Data Type:	Chromosomal effects battery in Aspergillus for chloral hydrate
HERO ID:	628831

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate. A CASRN was also provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity of the test substance was not reported. The test substance was indicated to be "lab grade".
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative (untreated) control groups were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design. Chloral hydrate was intended to be a positive control for this novel assay.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were reported; it was indicated that most methods were described in recent publications.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported (dissolved in water). Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (e.g., shown in Table 3).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	The exposure duration was given as a range (e.g., 3 to 4.5 hours or 6 to 7 hours) and differed based on dose (e.g., longer exposures for higher concentrations owing to slower germination). The study authors indicated that since effects did not increase with increasing dose, increased duration of exposure was used to approximate an increase in dose. However, the 2 hour time point was preferentially analyzed.

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Study Citation:	E. Käfer (1986). Tests which distinguish induced crossing-over and aneuploidy from secondary segregation in Aspergillus treated with chloral hydrate or gamma-rays Mutation Research, 164(3,3), 145-166
Data Type:	Chromosomal effects battery in Aspergillus for chloral hydrate
HERO ID:	628831

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups was reported (shown in Table 3). The assay was tested up to a toxic concentration (it was indicated that germ tubes were inhibited and results could not be analyzed at 80 mM).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design. The metabolite was tested directly.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The diploid strain used in the study was newly constructed; the haploid strain was obtained commercially. The genotype of the newly constructed diploid strain was shown in Figure 1 (with some details cited to other references).
	Metric 15:	Number per Group	Medium	× 1	2	The study stated that suspensions were diluted to 10ˆ6/mL. Treated conidia were plated at low densities (10 to 30 for diploids and 30 to 50 for haploids); based on sample sizes provided in Table 3, numbers of replicate plates were adequate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Low	× 2	6	Wide variations in sample size were reported in Table 3 (62 to 1424 for pre-germinated diploid cells and 106 to 1445 for haploid cells).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences in initial conditions were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	The study authors reported that, in an effort to increase sample size for the 2 hour time point, treated conidia were re-plated after being stored at 4 degrees C, which reduced their survival and increased numbers of abnormal colonies (i.e., in the 10 mM group).
Domain 7: Data Presentation and Analysis						

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Study Citation:	E. Käfer (1986). Tests which distinguish induced crossing-over and aneuploidy from secondary segregation in Aspergillus treated with chloral hydrate or gamma-rays Mutation Research, 164(3,3), 145-166
Data Type:	Chromosomal effects battery in Aspergillus for chloral hydrate
HERO ID:	628831

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	High	× 1	1	No statistical analysis was conducted (and not required). Data are shown as the incidence (%) of affected conidia and included sample size (% aneuploid in diploid cells, % conidia with altered chromosome numbers in haploid cells).
	Metric 23:	Data Interpretation	Medium	× 2	4	The criteria for a positive response was not clearly specified (other than increased numbers of aneuploid cells relative to controls).
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	This study was completed in conjunction with a measurement of cytotoxicity ("percent survival"); no additional information was provided.
	Metric 25:	Reporting of Data	High	× 2	2	All data are reported adequately. Data for negative findings were reported qualitatively.
Overall Quality Determination‡			Medium		1.7	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 116: **In vitro evaluation results for Keller and Heck 1988 for DNA binding study**

Study Citation:	D. A. Keller, H. Heck (1988). Mechanistic studies on chloral toxicity: Relationship to trichloroethylene carcinogenesis Toxicology Letters, 42(2,2), 183-191					
Data Type:	In vitro DNA binding for chloral/chloral hydrate					
HERO ID:	628835					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Low	× 2	6	The test substance was identified as chloral (trichloroacetaldehyde) as well as chloral hydrate. These two terms were used interchangeably throughout the article. Chloral is readily converted to chloral hydrate in the presence of water.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of chloral hydrate was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Untreated concurrent negative controls were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The preparation of the test substance was reported. The storage of the test substance was not reported (single dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	Number of exposure groups and dose spacing was reported and appropriate.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity and isolation and preparation methods for the F344 rat liver nuclei were reported.
	Metric 15:	Number per Group	High	× 1	1	The experiment was conducted with 3 replicates per exposure group.
Domain 5: Outcome Assessment						

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Study Citation:	D. A. Keller, H. Heck (1988). Mechanistic studies on chloral toxicity: Relationship to trichloroethylene carcinogenesis Toxicology Letters, 42(2,2), 183-191
Data Type:	In vitro DNA binding for chloral/chloral hydrate
HERO ID:	628835

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no differences reported in protocols across treatment groups. The acetaldehyde exposure duration was different from that of the chloral/chloral hydrate, but acetaldehyde was not considered for the present evaluation.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables were identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	The data were appropriately analyzed by one-way ANOVA.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria are consistent with current standards.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric is not applicable to the study design, as nuclei were used rather than whole cells.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported adequately.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 117: **In vitro evaluation results for Chang et al 1992 for DNA damage study in mouse hepatocytes**

Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Mouse hepatocyte DNA damage for TCA, DCA, and MCA
HERO ID:	628837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified as trichloroacetic acid (TCA), dichloroacetic acid (DCA), and monochloroacetic acid (MCA).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substances was reported (Sigma).
	Metric 3:	Test Substance Purity	Low	× 1	3	The purities of the test substances were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative controls were included. It was not specified whether the negative controls were treated with vehicle or left untreated.
	Metric 5:	Positive Controls	High	× 2	2	Concurrent positive controls (N-nitrosodimethylamine) were included in the study design.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The preparation of the test substance was reported. The storage of the test substance was not reported but is not expected to impact the results (short term assay)
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity in mM
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (4 hr) was reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	Number of exposure groups (3 plus control) and dose spacing was reported and appropriate.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This assay did not include an exogenous metabolic activation step, as the cells used were primary mouse hepatocytes.
Domain 4: Test Model						

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Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Mouse hepatocyte DNA damage for TCA, DCA, and MCA
HERO ID:	628837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The identity and isolation methods for the hepatocytes was reported and the model appropriate for the endpoint
	Metric 15:	Number per Group	High	× 1	1	The experiment was conducted with 3 replicates per exposure group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (alkaline unwinding) was described and appropriate for the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences among study group parameters that could influence outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	The data were appropriately analyzed by Dunnett's multiple comparison test.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria (statistical significance) are consistent with current standards.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The cytotoxicity of each experimental condition was tested with the LDH assay and results reported.
	Metric 25:	Reporting of Data	Medium	× 2	4	Variance was not reported (Figure 4).
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study.

Table 118: **In vitro evaluation results for Chang et al 1992 for DNA damage study in rat hepatocytes**

Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Rat hepatocyte DNA damage for TCA, DCA, MCA, and CH
HERO ID:	628837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified by name as trichloroacetic acid (TCA), dichloroacetic acid (DCA), monochloroacetic acid (MCA), and trichloroacetaldehyde (TCAA). It was reported that TCAA "in water exists as chloral hydrate." The vehicle for TCAA was water.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substances was reported (Sigma).
	Metric 3:	Test Substance Purity	Low	× 1	3	The purities of the test substances were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative controls were included. It was not specified whether the negative controls were treated with vehicle or left untreated.
	Metric 5:	Positive Controls	High	× 2	2	Concurrent positive controls (N-nitrosodiethylamine and methyl methansulfonate) were included in the study design.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The preparation of the test substance was reported. The storage of the test substance was not reported but is not expected to impact the results (short term assay)
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity in mM
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (4 hr) was reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	Number of exposure groups (3 plus control) and dose spacing was reported and appropriate.

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Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Rat hepatocyte DNA damage for TCA, DCA, MCA, and CH
HERO ID:	628837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This assay did not include an exogenous metabolic activation step, as the cells used were primary rat hepatocytes.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity and isolation methods for the hepatocytes was reported and the model appropriate for the endpoint
	Metric 15:	Number per Group	High	× 1	1	The experiment was conducted with 3 replicates per exposure group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (alkaline unwinding) was described and appropriate for the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences among study group parameters that could influence outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	The data were appropriately analyzed by Dunnett's multiple comparison test.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria (statistical significance) are consistent with current standards.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The cytotoxicity of each experimental condition was tested with the LDH assay and results reported.
	Metric 25:	Reporting of Data	Medium	× 2	4	Variance was not reported (Figure 3).
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

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Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Rat hepatocyte DNA damage for TCA, DCA, MCA, and CH
HERO ID:	628837

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 119: **In vitro evaluation results for Chang et al 1992 for DNA damage study in human lymphocytes**

Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Human T lymphoblast DNA damage for TCA, DCA, MCA, and CH
HERO ID:	628837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified by name as trichloroacetic acid (TCA), dichloroacetic acid (DCA), monochloroacetic acid (MCA), and trichloroacetaldehyde (TCAA). It was reported that TCAA "in water exists as chloral hydrate." The vehicle for TCAA was water.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substances was reported (Sigma).
	Metric 3:	Test Substance Purity	Low	× 1	3	The purities of the test substances were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative controls were included. It was not specified whether the negative controls were treated with vehicle or left untreated.
	Metric 5:	Positive Controls	High	× 2	2	Concurrent positive controls (methyl methanesulfonate) were included in the study design.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The preparation of the test substance was reported. The storage of the test substance was not reported but is not expected to impact the results (short term assay)
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity in mM
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (2 hr) was reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	Number of exposure groups (3 plus control) and dose spacing were reported and appropriate.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 4: Test Model						

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Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Human T lymphoblast DNA damage for TCA, DCA, MCA, and CH
HERO ID:	628837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The identity and commercial source of the cell line was reported and appropriate for the outcome.
	Metric 15:	Number per Group	High	× 1	1	The experiment was conducted with 3 replicates per exposure group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (alkaline unwinding) was described and appropriate for the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences among study group parameters that could influence outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	The data were appropriately analyzed by Dunnett's multiple comparison test.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria (statistical significance) are consistent with current standards.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The cytotoxicity of each experimental condition was tested with trypan blue exclusion and results were reported for each condition and dose
	Metric 25:	Reporting of Data	Medium	× 2	4	Variance was not reported (Figure 5).
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study.

Table 120: **In vitro evaluation results for Koch et al 1988 for S. cerevisiae reverse mutation study**

Study Citation:	R. Koch, R. Schlegelmilch, H. U. Wolf (1988). Genetic effects of chlorinated ethylenes in the yeast Saccharomyces cerevisiae Mutation Research, 206(2,2), 209-216
Data Type:	TCE mitotic gene conversion, reverse mutation and aneuploidy in yeast
HERO ID:	628846

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test material was identified by chemical name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified. Batch/lot number were not given, but the composition of the test material is not expected to vary.
	Metric 3:	Test Substance Purity	High	× 1	1	Analytical grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors acknowledged using a concurrent negative control group, but details regarding the negative control group were not reported. However, because test substances were pipetted directly into cell suspensions without vehicle, it is assumed that negative controls were untreated.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls (EMS) were used and responded appropriately.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described in detail and applicable to the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was added without dilution to the cell suspensions. This is considered to add uncertainty to the dosing, as direct dilution is less accurate than serial dilution due to human error or mechanical considerations (e.g. multiple pipettes used and potentially not calibrated appropriately).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations are reported as mM without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appropriate for the study type and outcome.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Concentrations were not justified, but were adequate to observe a dose-response (3 groups plus control).

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Study Citation:	R. Koch, R. Schlegelmilch, H. U. Wolf (1988). Genetic effects of chlorinated ethylenes in the yeast Saccharomyces cerevisiae Mutation Research, 206(2,2), 209-216
Data Type:	TCE mitotic gene conversion, reverse mutation and aneuploidy in yeast
HERO ID:	628846

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	High	× 1	1	Metabolic acrivation systems were well described.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was described with limited information (details cited elsewhere) and was routinely used.
	Metric 15:	Number per Group	Medium	× 1	2	Duplicate independent assays.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment reported and was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences were reported in initial conditions.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No differences were reported in the test model unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistics were not performed, but may not be necessary . Given values were from 1 representative test.
	Metric 23:	Data Interpretation	High	× 2	2	Scoring and/or evaluation criteria (i.e. meaning of colony colors and which were counted) were adequately reported.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were defined, but the methods of measurements were not fully described or reported.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group. Negative findings were reported quantitatively.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

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Study Citation:	R. Koch, R. Schlegelmilch, H. U. Wolf (1988). Genetic effects of chlorinated ethylenes in the yeast Saccharomyces cerevisiae Mutation Research, 206(2,2), 209-216
Data Type:	TCE mitotic gene conversion, reverse mutation and aneuploidy in yeast
HERO ID:	628846

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 121: **In vitro evaluation results for Crebelli et al 1985 for mutagenicity and mitotic segregation study**

Study Citation:	R. Crebelli, G. Conti, L. Conti, A. Carere (1985). Mutagenicity of trichloroethylene, trichloroethanol and chloral hydrate in Aspergillus nidulans Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 155(3,3), 105-111
Data Type:	mutagenicity and mitotic segregation in Aspergillus nidulans
HERO ID:	628852

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Trichloroethylene (TCE) and its metabolites, chloral hydrate (CH) and trichloroethanol (TCOH), were identified by chemical name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Sources were reported for TCE and metabolites. GC analysis was performed to identify impurities for TCE.
	Metric 3:	Test Substance Purity	High	× 1	1	TCE impurities <1%; CH 99% pure; trichloroethanol >95% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Negative controls were used for each experiment, but details regarding the negative control groups were not reported.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were used for each experiment and positive response were observed.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods were partially described and were cited in another publication, but appeared appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	In one experiment, TCE was dissolved in the media and solubility may be poor; however, at the concentrations used in this experiment, this is not considered to have resulted in precipitation or impacted results. Storage conditions were not described, but this is appropriate given the single-dose administration.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration appeared consistent across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported or estimated.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported for each experiment and was appropriate for the study type.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The concentrations were not justified, but the number of exposure groups and spacing of exposure levels were adequate to show results relevant to the outcome of interest.

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Study Citation:	R. Crebelli, G. Conti, L. Conti, A. Carere (1985). Mutagenicity of trichloroethylene, trichloroethanol and chloral hydrate in Aspergillus nidulans Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 155(3,3), 105-111
Data Type:	mutagenicity and mitotic segregation in Aspergillus nidulans
HERO ID:	628852

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13: Metabolic Activation	Not Rated	NA	NA	Typical metabolic activation systems were not used; however, endogenous metabolic conversion by A. nidulans was evaluated using the 'growth-mediated' technique. In addition, TCE metabolites were directly used in one of the experiments.
Domain 4: Test Model					
	Metric 14: Test Model	High	× 2	2	The test model was well-described and routinely used.
	Metric 15: Number per Group	High	× 1	1	Triplicate cultures were used.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported and sensitive for the outcome of interest.
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18: Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable for the outcome of interest.
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable for the outcome of interest.
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among initial study group parameters.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	There were no reported differences unrelated to exposure.
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	High	× 1	1	Statistical methods were reported and appropriate.
	Metric 23: Data Interpretation	Medium	× 2	4	Data evaluation criteria were partially described and cited to other references, but appeared to be appropriate.
	Metric 24: Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were defined, but the methods of measurements were not fully described or reported.
	Metric 25: Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group. Negative findings were reported quantitatively.
Overall Quality Determination[‡]		High		1.4	
Extracted		Yes			

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Study Citation:	R. Crebelli, G. Conti, L. Conti, A. Carere (1985). Mutagenicity of trichloroethylene, trichloroethanol and chloral hydrate in Aspergillus nidulans Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 155(3,3), 105-111
Data Type:	mutagenicity and mitotic segregation in Aspergillus nidulans
HERO ID:	628852

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 122: **In vitro evaluation results for Sora and Agostini Carbone 1987 for chromosome segregation study in S. cerevisiae**

Study Citation:	S. Sora, M. L. Agostini Carbone (1987). Chloral hydrate, methylmercury hydroxide and ethidium bromide affect chromosomal segregation during meiosis of Saccharomyces cerevisiae Mutation Research Letters, 190(1,1), 13-17
Data Type:	Chromosome segregation in yeast for chloral hydrate
HERO ID:	628916

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	CH was identified by chemical name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified. Batch/lot number was not reported, but the composition of the test material is not expected to vary.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	The study authors reported historical control means.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not used, but are likely not required by study type.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay procedures were described in other publications (Sora et al., 1982, 1983).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was described. No information on storage was reported.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were inferred and appeared consisitent across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	Assay procedures were described in other publications (Sora et al., 1982, 1983).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Concentration levels were not justified, but the number of exposure groups and spacing of exposure levels were adequate to show results relevant to the outcome of interest
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Assay procedures were described in other publications (Sora et al., 1982, 1983).
Domain 4: Test Model						
	Metric 14:	Test Model	Not Rated	NA	NA	The strains used were described in other publications (Sora et al., 1982, 1983).
	Metric 15:	Number per Group	Not Rated	NA	NA	Assay procedures were described in other publications (Sora et al., 1982, 1983).
Domain 5: Outcome Assessment						

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Study Citation:	S. Sora, M. L. Agostini Carbone (1987). Chloral hydrate, methylmercury hydroxide and ethidium bromide affect chromosomal segregation during meiosis of Saccharomyces cerevisiae Mutation Research Letters, 190(1,1), 13-17
Data Type:	Chromosome segregation in yeast for chloral hydrate
HERO ID:	628916

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methods reported and appeared sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Details of the outcome assessment methods were described in other publications (Sora et al., 1982, 1983).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial strain conditions were not reported for study groups.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analysis was not conducted, but may not be strictly required.
	Metric 23:	Data Interpretation	Low	× 2	6	Evaluation criteria were not reported.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were defined, but the methods of measurements were not fully described.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.
Overall Quality Determination‡			Unacceptable⋆⋆		2.1	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 123: **In vitro evaluation results for Vamvakas et al 1989 for unscheduled DNA synthesis study**

Study Citation:	Vamvakas, S., Dekant, W., Henschler, D. (1989). Assessment of unscheduled DNA synthesis in a cultured line of renal epithelial cells exposed to cysteine S-conjugates of haloalkenes and haloalkanes Mutation Research, 222(4,4), 329-335
Data Type:	Unscheduled DNA synthesis - DCVC (TCE metabolite)
HERO ID:	629909

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as S-(1,2-dichlorovinyl)-L-cysteine (DCVC), a metabolite of TCE
	Metric 2:	Test Substance Source	Medium	× 1	2	The synthesis and characterization of S-(1,2-dichlorovinyl)-L-cysteine (TCVC) was described in previously published studies (Dekant et al., 1986; Vadi et al., 1985)
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of test substance was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors report using a medium and solvent (0.5% MeOH) control.
	Metric 5:	Positive Controls	High	× 2	2	Nitroquinoline oxide (NQO) was used as a positive control and gave expected results.
	Metric 6:	Assay Procedures	High	× 1	1	Study authors described the methods and procedures used for the test and they were applicable for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was described as dissolved in MeOH 30 to 60 seconds before incubation to avoid decomposition in solution.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were reported to be administered consistently across treated and control groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentrations were reported in the results.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (24 hours).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of dose groups and spacing was not justified by the study authors, however the number of exposure groups and spacing were adequate to show results relative to the outcome of interest.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable
Domain 4: Test Model						

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Study Citation: Vamvakas, S., Dekant, W., Henschler, D. (1989). Assessment of unscheduled DNA synthesis in a cultured line of renal epithelial cells exposed to cysteine S-conjugates of haloalkenes and haloalkanes Mutation Research, 222(4,4), 329-335
Data Type: Unscheduled DNA synthesis - DCVC (TCE metabolite)
HERO ID: 629909

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	The test model (LLC-PK1 cells) was reported with limited descriptive information. The cells were obtained from a commercial source (American Type Culture Collection). The test model is appropriate for the outcome of interest.
	Metric 15:	Number per Group	Medium	× 1	2	The number of cells was reported (2 x 10+6); Determinations made in quadruplicate and experiments were repeated at least 2 times.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were reported and appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across the controls and treated groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	3x10+5 cells were plated on each culture dish determinations were made in quadruplicate and experiments were repeated at least 2 times.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This method is not applicable to the outcome.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial quality of cells exposed and lot of test substance was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Significance of changes in UDS was noted; however, methods for statistical analysis were not clearly described; results shown in a figure indicate a mean and SD from 2 independent experiments; independent statistical analysis could be performed.
	Metric 23:	Data Interpretation	Low	× 2	6	Scoring and evaluation criteria were not reported; however, the induction of UDS is evaluated as a change from the control at 24 hours.
	Metric 24:	Cytotoxicity Data	High	× 1	1	There was a determination of cell viability as indicated by lactate dehydrogenase release in the medium.
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcomes were presented for each exposure group as a mean and SD.
Overall Quality Determination‡			High		1.5	

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Study Citation:	Vamvakas, S., Dekant, W., Henschler, D. (1989). Assessment of unscheduled DNA synthesis in a cultured line of renal epithelial cells exposed to cysteine S-conjugates of haloalkenes and haloalkanes Mutation Research, 222(4,4), 329-335
Data Type:	Unscheduled DNA synthesis - DCVC (TCE metabolite)
HERO ID:	629909

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i \left(\text{Metric Score}_i \times \text{MWF}_i\right) / \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 124: **In vitro evaluation results for Wang et al 2001 for micronucleus assay study**

Study Citation:	J. L. Wang, W. L. Chen, S. Y. Tsai, P. Y. Sung, R. N. Huang (2001). An in vitro model for evaluation of vaporous toxicity of trichloroethylene and tetrachloroethylene to CHO-K1 cells Chemico-Biological Interactions, 137(2,2), 139-154
Data Type:	Micronucleus assay for TCE
HERO ID:	629916

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported. Although a batch/lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported (99%); purity was such that effects were likely due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study authors reported using concurrent negative controls; the type of control used (untreated or solvent-only) was not clearly specified.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control is not strictly required by study type. Test substances used in the assay produced positive, dose-related responses (indicative that the assay was effective).
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were briefly described and cited to another publication (Fenech 1993).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Storage was not reported (but not expected to impact the study results). The study indicated that the test substance was added as a liquid to a central (open) glass dish and allowed to evaporate and dissolve in the surrounding medium (closed, but not sealed petri dish containing cultured cells). Although there was evidence that the test substance volatilized from the test vessels, actual test substance concentrations (while extremely low) were measured by gas chromatography.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	It was inferred that exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses (after 24 hours exposure) could be estimated from Figure 2.

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Study Citation:	J. L. Wang, W. L. Chen, S. Y. Tsai, P. Y. Sung, R. N. Huang (2001). An in vitro model for evaluation of vaporous toxicity of trichloroethylene and tetrachloroethylene to CHO-K1 cells Chemico-Biological Interactions, 137(2,2), 139-154
Data Type:	Micronucleus assay for TCE
HERO ID:	629916

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	The exposure duration was reported (24 hours), but exceeded the recommendation for this study type.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of dose groups was reported (3 plus controls) and appropriate. However, owing to the volatility of the test substance, actual test concentrations fell into a narrow (less than 2-fold) range. In addition, cytotoxicity was excessive (particularly at the two highest tested concentrations).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was reported (CHO-K1 cells); this cell type is routinely used in genotoxicity tests. However, the test model was identified with little to no additional information (e.g., source).
	Metric 15:	Number per Group	High	× 1	1	The study indicated that results represented four independent experiments.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology appeared to be appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	The outcome assessment was inferred to be consistent across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The study indicated that 500 binucleated cells per dish were examined (i.e., 2000 cells/dose group).
	Metric 19:	Blinding of Assessors	High	× 1	1	It was indicated that the dishes were blindly coded.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding differences were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistics were reported and were appropriate for the study type and data presented. The data shown graphically (means +/-SD) are also sufficient for independent analyses.
	Metric 23:	Data Interpretation	Medium	× 2	4	While not explicitly specified, the statistical significance and dose-relatedness of the response appeared to be the criteria for a positive response.

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Study Citation:	J. L. Wang, W. L. Chen, S. Y. Tsai, P. Y. Sung, R. N. Huang (2001). An in vitro model for evaluation of vaporous toxicity of trichloroethylene and tetrachloroethylene to CHO-K1 cells Chemico-Biological Interactions, 137(2,2), 139-154
Data Type:	Micronucleus assay for TCE
HERO ID:	629916

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Cytotoxicity Data	Medium	× 1	2	Cytotoxicity methods were briefly reported (i.e., cell count using a hematocytometer).
	Metric 25: Reporting of Data	High	× 2	2	Data was reported for each exposure group.
Overall Quality Determination‡		Medium		1.8	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 125: **In vitro evaluation results for Harrington-Brock et al 1998 for clastogenic effects in mouse lymphoma cells**

Study Citation:	K. Harrington-Brock, C. L. Doerr, M. M. Moore (1998). Mutagenicity of three disinfection by-products: Di- and trichloroacetic acid and chloral hydrate in L5178Y(+/-) --3.7.2C mouse lymphoma cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 413(3,3), 265-276
Data Type:	Clastogenic effects for TCE metabolites (CH and DCA)
HERO ID:	632659

Domain	Metric		Rating†	MWF*	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE metabolites were identified by name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Source manufacturers were reported. Although batch/lot numbers were not provided, the test substances are not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using concurrent negative controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	The study authors did not report using a positive control (not absolute requirement by study type). For chromosomal aberrations, test substances used in the assay generated (at least weakly) positive dose-responses (indicating the efficacy of the assay to detect a positive response).
	Metric 6:	Assay Procedures	Low	× 1	3	Assay methods and procedures were not well-described and were partially cited to other publications.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported (dissolved in culture medium for DCA; dissolved in saline for CH). Storage was not reported (but not likely to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration appeared to be consistent across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The duration of exposure was reported.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of exposure groups was reported (2 doses plus controls), but was lower than the number typically used for studies of this type.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.

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Study Citation:	K. Harrington-Brock, C. L. Doerr, M. M. Moore (1998). Mutagenicity of three disinfection by-products: Di- and trichloroacetic acid and chloral hydrate in L5178Y(+/-) --3.7.2C mouse lymphoma cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 413(3,3), 265-276
Data Type:	Clastogenic effects for TCE metabolites (CH and DCA)
HERO ID:	632659

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 4: Test Model					
Metric 14:	Test Model	Medium	× 2	4	The test model (L5178Y mouse lymphoma cells) was reported and is routinely used in genotoxicity studies (including micronucleus assays). The source of cells was not reported.
Metric 15:	Number per Group	Low	× 1	3	Single cultures appeared to have been used.
Domain 5: Outcome Assessment					
Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome methodology was reported and addressed the intended outcomes of interest.
Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
Metric 18:	Sampling Adequacy	Low	× 2	6	Sampling was 100 metaphases/concentration for CAs and 1000 binucleated cells/concentration for MNs; both are less than what is recommended for studies of these types (i.e., 300 metaphases and 2000 binucleated cells/concentration).
Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was reported for the chromosomal aberrations assay (i.e., slides were coded). Blinding was not explicitly specified for MN.
Domain 6: Confounding / Variable Control					
Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no reported confounding variables in the test design.
Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables unrelated to the test substance were reported.
Domain 7: Data Presentation and Analysis					
Metric 22:	Data Analysis	Not Rated	NA	NA	Data were were for one culture/concentration. Although the study cites "significant" effects in the text, it does not appear that any statistics were performed.
Metric 23:	Data Interpretation	Medium	× 2	4	The study provided some indication that the criteria for a positive response was a two-fold increase and/or a dose-related response. The study also indicated that a two-fold increase in response relative to the historical mean for all negative controls (not clearly specified) was an additional criterion.

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Study Citation:	K. Harrington-Brock, C. L. Doerr, M. M. Moore (1998). Mutagenicity of three disinfection by-products: Di- and trichloroacetic acid and chloral hydrate in L5178Y(+/-) --3.7.2C mouse lymphoma cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 413(3,3), 265-276
Data Type:	Clastogenic effects for TCE metabolites (CH and DCA)
HERO ID:	632659

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity methods were largely cited to another publication (Clive and Spector 1975). Cytotoxicity (measured as relative survival) was measured concurrently.
	Metric 25: Reporting of Data	High	× 2	2	Data was reported by exposure group (Table 2).
Overall Quality Determination‡		Medium		1.8	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 126: **In vitro evaluation results for Harrington-Brock et al 1998 for mutagenicity effects in mouse lymphoma cells**

Study Citation:	K. Harrington-Brock, C. L. Doerr, M. M. Moore (1998). Mutagenicity of three disinfection by-products: Di- and trichloroacetic acid and chloral hydrate in L5178Y(+/-) --3.7.2C mouse lymphoma cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 413(3,3), 265-276
Data Type:	Mutagenicity for TCE metabolites (CH, DCA and TCA)
HERO ID:	632659

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE metabolites were identified by name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Source manufacturers were reported. Although batch/lot numbers were not provided, the test substances are not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using concurrent negative controls.
	Metric 5:	Positive Controls	High	× 2	2	Appropriate positive control substances were used. Positive control substances elicited positive responses. It is noted that in one of the assays (TCA experiment #1; see Table 1), cytotoxicity was excessive (4% survival).
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described in adequate detail. Only minor details regarding cell culture maintenance were cited to another publication.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported (dissolved in culture medium for TCA and DCA; dissolved in saline for CH). Storage was not reported (but not likely to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration appeared to be consistent across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported (4 hours) and appropriate for the study type.

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Study Citation:	K. Harrington-Brock, C. L. Doerr, M. M. Moore (1998). Mutagenicity of three disinfection by-products: Di- and trichloroacetic acid and chloral hydrate in L5178Y(+/-) --3.7.2C mouse lymphoma cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 413(3,3), 265-276
Data Type:	Mutagenicity for TCE metabolites (CH, DCA and TCA)
HERO ID:	632659

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups was reported (Table 1). Doses were presumably selected based on previous genotoxicity and/or the results of concurrent cytotoxicity tests. Doses that elicited excessive cytotoxicity were not presented in the graphical results (Figure 1). Although there were a sufficient number of analyzable concentrations (and dose-responses were observed), the dose range in some cases appeared narrow (less than a three-fold change between lowest and highest doses).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type. The study directly tested the metabolites of TCE. However, one of the metabolites (TCA, sodium salt) was tested in the presence of activation; the source of S9 (a manufacturer) was reported without additional details (with respect to type, final concentration).
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model (L5178Y mouse lymphoma cells) was reported and is routinely used in studies of this type. The source of cells was not reported.
	Metric 15:	Number per Group	High	× 1	1	The study indicated that in lieu of using replicate cultures, multiple independent experiments were performed (at least two).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome methodology was reported and addressed the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The study authors indicated that pH was monitored (at the start and at the end of treatment) in some assays; as differences in pH (low pH) has been presumed to be the reason for positive results. Data for pH was provided for some doses in some assays, at the beginning of exposure only. The authors indicated that pH data show that effects were due to the chemical itself rather than changes in pH.

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Study Citation:	K. Harrington-Brock, C. L. Doerr, M. M. Moore (1998). Mutagenicity of three disinfection by-products: Di- and trichloroacetic acid and chloral hydrate in L5178Y(+/-) --3.7.2C mouse lymphoma cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 413(3,3), 265-276
Data Type:	Mutagenicity for TCE metabolites (CH, DCA and TCA)
HERO ID:	632659

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data were not reported for outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analyses were not performed, and not required by study type.
	Metric 23:	Data Interpretation	Medium	× 2	4	The study provided some indication that the criteria for a positive response was a two-fold increase and/or a dose-related response.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity methods were largely cited to another publication (Clive and Spector 1975). Cytotoxicity (measured as relative survival) was measured concurrently, and reportedly included measures of growth in suspension and cloning phases.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group (Table 1).
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 127: **In vitro evaluation results for Shimada et al 1985 for bacterial reverse mutation study**

Study Citation:	T. Shimada, A. F. Swanson, P. Leber, G. M. Williams (1985). Activities of chlorinated ethane and ethylene compounds in the Salmonella/rat microsome mutagenesis and rat hepatocyte/DNA repair assays under vapor phase exposure conditions Cell Biology and Toxicology, 1(3,3), 159-179
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	632848

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by established nomenclature.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified. A batch/lot number was not given, but the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be >99.5% pure (99.98% for low-stabilized and 99.5% for stabilized form).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using non-exposed controls.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were used (vinyl chloride) and responded appropriately.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and also cited in other publications, but appeared to be appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage were well-described and appropriate for the test substance.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups in a scientifically sound manner.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Vapor concentrations were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration for the bacterial mutation assay was reported to be 18h with a total incubation time of 48-72h.

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Study Citation:	T. Shimada, A. F. Swanson, P. Leber, G. M. Williams (1985). Activities of chlorinated ethane and ethylene compounds in the Salmonella/rat microsome mutagenesis and rat hepatocyte/DNA repair assays under vapor phase exposure conditions Cell Biology and Toxicology, 1(3,3), 159-179
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	632848

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	Cytotoxicity data were used to justify analyzable exposure concentrations. The number of exposure groups was not explicitly specified (3 doses were shown in Table 5). A range of doses from 1% to 7.5% was reported in the legend for Table 5, with some doses not shown in the table owing to total cell death.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The presence of a commonly used metabolic activation system was reported (S9 from Aroclor 1254 induced rats); however, some details regarding type, composition mix, concentration, or quality control information were not described.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test models were reported along with limited descriptive information and were routinely used for the outcomes of interest.
	Metric 15:	Number per Group	High	× 1	1	There were 3 replicates for each experiment. Based on Table 5, it appears that 2 experiments were conducted using strain TA 100 without activation (presumably all doses/forms), and 8 experiments for TA 100 controls (i.e., spontaneous revertants).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methods addressed and were sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment were reported and were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcomes of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences were reported among initial study group parameters.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	There were no reported differences among the study replicates or groups in test models unrelated to exposure.
Domain 7: Data Presentation and Analysis						

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Study Citation:	T. Shimada, A. F. Swanson, P. Leber, G. M. Williams (1985). Activities of chlorinated ethane and ethylene compounds in the Salmonella/rat microsome mutagenesis and rat hepatocyte/DNA repair assays under vapor phase exposure conditions Cell Biology and Toxicology, 1(3,3), 159-179
Data Type:	Bacterial reverse mutation for TCE
HERO ID:	632848

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analyses were not performed, but may not be strictly required.
	Metric 23:	Data Interpretation	High	× 2	2	Study authors reported the evaluation criteria for determining a positive outcome which were consistent with established practices (more than 2-fold increase over controls).
	Metric 24:	Cytotoxicity Data	High	× 1	1	The methods for measuring cytotoxicity were clearly described and commonly used for assessment.
	Metric 25:	Reporting of Data	Low	× 2	6	Data for exposure-related findings were not shown for each study group, but results were described in the text (Table 5 was missing information and negative results were reported qualitatively). Positive results that were not shown included effects for stabilized TCE in strain TA 100 with activation, and effects in strain TA 1535 with and without activation. It is also noted that Table 5 does not clearly indicate the concentrations that correspond to each row (but this information can be inferred from the text).
Overall Quality Determination‡			~~High~~ ⟶ Medium§		~~1.5~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "The study provides useful information with respect to mutagenicity data. However, data were not presented adequately. Data were presented for S typhimurium strain TA 100 only without activation (and the table did not label the corresponding concentrations); positive results were not shown for strain TA 100 with activation or strain TA 1535 with and without activation (fold-changes reported in text). The conditions of the study produced substantial toxicity at many of the doses that were evaluated."

Table 128: **Animal toxicity evaluation results for Rossi et al 1983 for host-mediated mutagenicity study**

Study Citation:	A. M. Rossi, L. Migliore, R. Barale, N. Loprieno (1983). In vivo and in vitro mutagenicity studies of a possible carcinogen, trichloroethylene, and its two stabilizers, epichlorohydrin and 1,2-epoxybutane Teratogenesis, Carcinogenesis, and Mutagenesis, 3(1,1), 75-87
Data Type:	Host mediated assay for mutagenicity
HERO ID:	18895

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was reported as TCE pure grade
	Metric 2:	Test Substance Source	High	× 1	1	Obtained from Montedison, Occupational Medicine and Industrial service, Milano, Italy. Identity was verified by GC
	Metric 3:	Test Substance Purity	High	× 1	1	99.98% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle (corn oil) control
	Metric 5:	Positive Controls	High	× 1	1	NDMA, MMS are positive controls and were listed in the table 7
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation to study groups was not reported
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation in corn oil was inferred corn oil controls described in Table VII. Storage was not described.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Administration of the test substance was consistent across study groups with the same gavage volume and frequency.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Single gavage dose of 0 or 2 g/kg.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Administration of the test substance by gavage with IV and IP administration were 4 and 6 hours, respectively and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Number of dose groups is reported in table 7; Single dose was reported and was adequate for this study type but no justification was reported.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was appropriate for this study type
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Male CD1 x C57BL hybrid mice were used but source was not reported
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were not reported
	Metric 15:	Number per Group	High	× 1	1	Number of animals was reported in table 7 and was appropriate for the outcome.
Domain 5: Outcome Assessment						

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Study Citation:	A. M. Rossi, L. Migliore, R. Barale, N. Loprieno (1983). In vivo and in vitro mutagenicity studies of a possible carcinogen, trichloroethylene, and its two stabilizers, epichlorohydrin and 1,2-epoxybutane Teratogenesis, Carcinogenesis, and Mutagenesis, 3(1,1), 75-87
Data Type:	Host mediated assay for mutagenicity
HERO ID:	18895

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for this endpoint.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across study groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for this study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for this study type
	Metric 20:	Negative Control Response	High	× 1	1	Low response was observed in the negative control and was adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial, body weight, food, and water consumption were not reported for each group.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were not reported for the in vivo study, but data provided is sufficient for statistical analysis
	Metric 24:	Reporting of Data	High	× 2	2	Quantitative data were reported for all groups in table 7. No summary data was presented
Overall Quality Determination‡			High		1.6	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 129: **In vitro evaluation results for Shimada et al 1985 for DNA repair study in rat hepatocytes**

Study Citation:	T. Shimada, A. F. Swanson, P. Leber, G. M. Williams (1985). Activities of chlorinated ethane and ethylene compounds in the Salmonella/rat microsome mutagenesis and rat hepatocyte/DNA repair assays under vapor phase exposure conditions Cell Biology and Toxicology, 1(3,3), 159-179
Data Type:	DNA repair in rat hepatocytes for TCE
HERO ID:	632848

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by established nomenclature.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified. A batch/lot number was not given, but the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be >99.5% pure (99.98% for low-stabilized and 99.5% for stabilized form).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using non-exposed controls. Fluorene was also used as a negative control in the conventional (liquid) assay.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were used (2-acetyl amino fluorene for liquid assay; monochloroethylene for vapor exposure).
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and also cited in other publications, but appeared to be appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage were well-described and appropriate for the test substance.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups in a scientifically sound manner.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Vapor and liquid concentrations were reported (as %).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration was reported to be 3 hours or 18 hours. The study provided a rationale for the duration of exposure (e.g., based on a preliminary dose-finding study using monochloroethylene). However, reducing the duration of exposure to 3 hours did not reduce cytotoxicity.

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Study Citation:	T. Shimada, A. F. Swanson, P. Leber, G. M. Williams (1985). Activities of chlorinated ethane and ethylene compounds in the Salmonella/rat microsome mutagenesis and rat hepatocyte/DNA repair assays under vapor phase exposure conditions Cell Biology and Toxicology, 1(3,3), 159-179
Data Type:	DNA repair in rat hepatocytes for TCE
HERO ID:	632848

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	Doses were based on a preliminary dose-finding study. However, test substances used in the assay were more cytotoxic than monochloroethylene (used in the preliminary assay). In the vapor assay with 18 hours exposure, there was substantial (nearly 100%) toxicity at all doses used for the low-stabilized form and complete toxicity at the highest dose for the stabilized form; there was only moderate toxicity after 3 hours exposure. In the conventional (liquid) assay with 18 hours exposure, there was complete toxicity at the highest dose for the low-stabilized form (leaving only one analyzable dose) and at the highest dose for the stabilized form. After 3 hours exposure, there was nearly 100% toxicity at the two highest doses for the low-stabilized form and complete toxicity at the highest dose for the stabilized form.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Exogenous metabolic activation was not needed for rat hepatocytes.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test models were reported along with limited descriptive information and were routinely used for the outcomes of interest.
	Metric 15:	Number per Group	High	× 1	1	The study indicated that 3 replicates were used.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methods addressed and were sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment were reported and were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcomes of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences reported among initial study group parameters.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	There were no reported differences among the study replicates or groups in test models unrelated to exposure.
Domain 7: Data Presentation and Analysis						

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Study Citation:	T. Shimada, A. F. Swanson, P. Leber, G. M. Williams (1985). Activities of chlorinated ethane and ethylene compounds in the Salmonella/rat microsome mutagenesis and rat hepatocyte/DNA repair assays under vapor phase exposure conditions Cell Biology and Toxicology, 1(3,3), 159-179
Data Type:	DNA repair in rat hepatocytes for TCE
HERO ID:	632848

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analyses were not performed, but may not be strictly required. Data provided would be amenable to statistical analyses.
	Metric 23:	Data Interpretation	Low	× 2	6	The study indicated that the criteria for a positive response was when the minimum net grain count exceeded 5 nuclei and was "significantly" above controls in 2 experiments. The rationale for this cut-off and the criteria for a significant response (in the absence of statistical analyses) was not clearly specified.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The methods for measuring cytotoxicity were clearly described and commonly used for assessment.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 130: **In vitro evaluation results for Parry et al 1996 for cell transformation study**

Study Citation:	J. M. Parry, E. M. Parry, R. Bourner, A. Doherty, S. Ellard, J. O'Donovan, B. Hoebee, J. M. de Stoppelaar, G. R. Mohn, A. Onfelt, A. Renglin, N. Schultz, C. Soderpalm-Berndes, K. G. Jensen, M. Kirsch-Volders, A. Elhajouji, P. Van Hummelen, F. Degrassi, A. Antoccia, D. Cimini, M. Izzo, C. Tanzarella, I. D. Adler, U. Kliesch, G. Schriever-Schwemmer, P. Gasser, R. Crebelli, A. Carere, C. Andreoli, R. Benigni, P. Leopardi, F. Marcon, Z. Zinjo, A. T. Natarajan, J. Boei, A. Kappas, G. Voutsinas, F. E. Zarani, A. Patrinelli, F. Pachierotti, C. Tiveron, P. Hess (1996). The detection and evaluation of aneugenic chemicals Mutation Research, 353(1-2,1-2), 11-46
Data Type:	Cell transformation in hamster dermal cells (chloral hydrate)
HERO ID:	657901

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chloral hydrate was identified by established nomenclature.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substances were not identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Solvent controls were used.
	Metric 5:	Positive Controls	High	× 2	2	Concurrent positive control was used with expected results (N-methyl-N'-nitro-N-nitrosourea).
	Metric 6:	Assay Procedures	High	× 1	1	The methods were well described and appropriate for the endpoint.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric does not apply to these data.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage was not reported.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Consistent exposure across groups was inferred from the text; however, details were not reported.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was for 3-24 h.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Concentrations were not justified, but a dose response relationship was apparent.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Cells were metabolically active.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Dermal hamster cells are not routinely used for cell transformation.
	Metric 15:	Number per Group	High	× 1	1	3 flasks used per concentration.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The method appears sensitive for the outcome of interest.

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Study Citation:	J. M. Parry, E. M. Parry, R. Bourner, A. Doherty, S. Ellard, J. O'Donovan, B. Hoebee, J. M. de Stoppelaar, G. R. Mohn, A. Onfelt, A. Renglin, N. Schultz, C. Soderpalm-Berndes, K. G. Jensen, M. Kirsch-Volders, A. Elhajouji, P. Van Hummelen, F. Degrassi, A. Antoccia, D. Cimini, M. Izzo, C. Tanzarella, I. D. Adler, U. Kliesch, G. Schriever-Schwemmer, P. Gasser, R. Crebelli, A. Carere, C. Andreoli, R. Benigni, P. Leopardi, F. Marcon, Z. Zinjo, A. T. Natarajan, J. Boei, A. Kappas, G. Voutsinas, F. E. Zarani, A. Patrinelli, F. Pachierotti, C. Tiveron, P. Hess (1996). The detection and evaluation of aneugenic chemicals Mutation Research, 353(1-2,1-2), 11-46
Data Type:	Cell transformation in hamster dermal cells (chloral hydrate)
HERO ID:	657901

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consitently across groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric does not apply to this outcome.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to this outcome.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Minimal details were providing regarding initial test conditions.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Differences unrelated to exposure were not reported among groups.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Unacceptable	× 1	4	Statistical analysis was not reported and variance data were not provided (independent analyses not possible).
	Metric 23:	Data Interpretation	Low	× 2	6	Scoring and/or evaluation criteria were not reported.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Relative cloning efficiency was reduced at the 3 highest concentrations.
	Metric 25:	Reporting of Data	Medium	× 2	4	Data were reported as transformation frequency/106 seeded cells.
Overall Quality Determination‡			Unacceptable⋆⋆		1.9	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 131: **In vitro evaluation results for Parry et al 1996 for micronuclei study**

Study Citation:	J. M. Parry, E. M. Parry, R. Bourner, A. Doherty, S. Ellard, J. O'Donovan, B. Hoebee, J. M. de Stoppelaar, G. R. Mohn, A. Onfelt, A. Renglin, N. Schultz, C. Soderpalm-Berndes, K. G. Jensen, M. Kirsch-Volders, A. Elhajouji, P. Van Hummelen, F. Degrassi, A. Antoccia, D. Cimini, M. Izzo, C. Tanzarella, I. D. Adler, U. Kliesch, G. Schriever-Schwemmer, P. Gasser, R. Crebelli, A. Carere, C. Andreoli, R. Benigni, P. Leopardi, F. Marcon, Z. Zinjo, A. T. Natarajan, J. Boei, A. Kappas, G. Voutsinas, F. E. Zarani, A. Patrinelli, F. Pachierotti, C. Tiveron, P. Hess (1996). The detection and evaluation of aneugenic chemicals Mutation Research, 353(1-2,1-2), 11-46
Data Type:	Micronuclei in human lymphoblastoid cells (TCE, chloral hydrate)
HERO ID:	657901

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE and chloral hydrate were identified by established nomenclature.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substances were not identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Study authors acknowledged using a concurrent negative control group (see Tables 15 and 17), but details regarding the negative control group were not reported (i.e., vehicle or untreated).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable; methodology under development.
	Metric 6:	Assay Procedures	Low	× 1	3	The methods were not well described. Micronuclei induction in binucleated cells of human lymphoblastoid cell lines.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric does not apply to these data.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage was not reported.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Consistent exposure across groups was inferred from the text; however, details were not reported.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Unacceptable	× 2	8	Exposure duration was not reported.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Concentrations were not justified, but a dose response relationship was apparent.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Cells lines were described as metabolically active.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	Cell line names were reported with no\few additional details.

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Study Citation:	J. M. Parry, E. M. Parry, R. Bourner, A. Doherty, S. Ellard, J. O'Donovan, B. Hoebee, J. M. de Stoppelaar, G. R. Mohn, A. Onfelt, A. Renglin, N. Schultz, C. Soderpalm-Berndes, K. G. Jensen, M. Kirsch-Volders, A. Elhajouji, P. Van Hummelen, F. Degrassi, A. Antoccia, D. Cimini, M. Izzo, C. Tanzarella, I. D. Adler, U. Kliesch, G. Schriever-Schwemmer, P. Gasser, R. Crebelli, A. Carere, C. Andreoli, R. Benigni, P. Leopardi, F. Marcon, Z. Zinjo, A. T. Natarajan, J. Boei, A. Kappas, G. Voutsinas, F. E. Zarani, A. Patrinelli, F. Pachierotti, C. Tiveron, P. Hess (1996). The detection and evaluation of aneugenic chemicals Mutation Research, 353(1-2,1-2), 11-46
Data Type:	Micronuclei in human lymphoblastoid cells (TCE, chloral hydrate)
HERO ID:	657901

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	Number of replicates not reported; assumed to be single.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Due to incomplete reporting, it was unclear whether methods were sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details regarding the outcome assessment were not reported.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	2000 binucleate cells per concentration (except at highest concentrations)
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to this metric.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Minimal details were providing regarding initial test conditions.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Differences unrelated to exposure were not reported among groups.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Unacceptable	× 1	4	Statistical analysis was not reported and variance data were not provided (independent analyses not possible).
	Metric 23:	Data Interpretation	Low	× 2	6	Scoring and/or evaluation criteria were not reported.
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.
	Metric 25:	Reporting of Data	Medium	× 2	4	Data were preported as % micronucleated cells.
Overall Quality Determination‡			Unacceptable⋆⋆		2.6	
Extracted			No			

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Study Citation:	J. M. Parry, E. M. Parry, R. Bourner, A. Doherty, S. Ellard, J. O'Donovan, B. Hoebee, J. M. de Stoppelaar, G. R. Mohn, A. Onfelt, A. Renglin, N. Schultz, C. Soderpalm-Berndes, K. G. Jensen, M. Kirsch-Volders, A. Elhajouji, P. Van Hummelen, F. Degrassi, A. Antoccia, D. Cimini, M. Izzo, C. Tanzarella, I. D. Adler, U. Kliesch, G. Schriever-Schwemmer, P. Gasser, R. Crebelli, A. Carere, C. Andreoli, R. Benigni, P. Leopardi, F. Marcon, Z. Zinjo, A. T. Natarajan, J. Boei, A. Kappas, G. Voutsinas, F. E. Zarani, A. Patrinelli, F. Pachierotti, C. Tiveron, P. Hess (1996). The detection and evaluation of aneugenic chemicals Mutation Research, 353(1-2,1-2), 11-46
Data Type:	Micronuclei in human lymphoblastoid cells (TCE, chloral hydrate)
HERO ID:	657901

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 132: **In vitro evaluation results for Mally et al 2006 for cell transformation study**

Study Citation:	A. Mally, C. Walker, J. Everitt, W. Dekant, S. Vamvakas (2006). Analysis of renal cell transformation following exposure to trichloroethene in vivo and its metabolite S-(dichlorovinyl)-L-cysteine in vitro Toxicology, 224(1-2,1-2), 108-118
Data Type:	Cell transformation for DCVC
HERO ID:	700373

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as S-(dichlorovinyl)-L-cysteine (DCVC).
	Metric 2:	Test Substance Source	High	× 1	1	The study indicated that chemicals were purchased from Sigma-Aldrich unless otherwise specified (there was no other indication for DCVC).
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of DCVC was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using concurrent negative (medium-only) controls.
	Metric 5:	Positive Controls	Medium	× 2	4	The study authors reported using a positive control group (i.e., cells treated with N-methyl-N'-nitro-N-nitrosoguanidin [MNNG]). This substance was shown to transform this cell line in a previous study (Horesovsky et al. 1994). The positive controls reportedly elicited a positive response (quantitative data were not shown; a representative plate showing transformed colonies was shown in Figure 3).
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were reported and appeared adequate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of test substance was reported (i.e., dissolved in water). Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Information on exposure administration was reported and consistency of administration is inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported (24 hours). The study authors provided a rationale for an extended exposure time relative to MNNG (i.e., time required for bioactivation of DCVC).

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Study Citation:	A. Mally, C. Walker, J. Everitt, W. Dekant, S. Vamvakas (2006). Analysis of renal cell transformation following exposure to trichloroethene in vivo and its metabolite S-(dichlorovinyl)-L-cysteine in vitro Toxicology, 224(1-2,1-2), 108-118
Data Type:	Cell transformation for DCVC
HERO ID:	700373

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The study indicated that 10 uM DCVC was used for the cell transformation assay (i.e., a single dose). The dose was presumably selected based on cytotoxicity.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type. A metabolite of TCE (DCVC) was used directly in this assay.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was described but is not routinely used for the outcome of interest. This particular cell line (carrying the Tsc-2 germline mutation) is uniquely susceptible to cell transformation.
	Metric 15:	Number per Group	High	× 1	1	Based on data shown in Table 3, it appears that at least 7 experiments were performed. In each experiment, cells were seeded at 10,000 cells/60 mm dish (20-30 dishes per group).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology addressed the intended outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in test design or procedures were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables unrelated to treatment were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical evaluations were described in the methods, but were not applied to transformation data. Statistics are not strictly required by study type.
	Metric 23:	Data Interpretation	Low	× 2	6	The study results stated that the DCVC transformation frequency "was variable, but consistently higher than background (Figure 3)." The transformed colonies were also presumably compared to MNNG (positive control) transformants (morphologically).

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Study Citation:	A. Mally, C. Walker, J. Everitt, W. Dekant, S. Vamvakas (2006). Analysis of renal cell transformation following exposure to trichloroethene in vivo and its metabolite S-(dichlorovinyl)-L-cysteine in vitro Toxicology, 224(1-2,1-2), 108-118
Data Type:	Cell transformation for DCVC
HERO ID:	700373

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 24:	Cytotoxicity Data	High	× 1	1	The study reported that relative survival was determined as (CFE treated dishes)/(CFE control dishes). Relative survival values were reported for each experiment in Table 3.
	Metric 25:	Reporting of Data	Medium	× 2	4	For each experiment, the colony forming efficiency (%), relative survival (%), cell number x 10ˆ4, number of colonies, and transformation efficiency (%) were reported for DCVC treated cells and controls (positive control data not shown).
Overall Quality Determination‡			Medium		1.7	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 133: **In vitro evaluation results for Mally et al 2006 for mutagenicity study**

Study Citation:	A. Mally, C. Walker, J. Everitt, W. Dekant, S. Vamvakas (2006). Analysis of renal cell transformation following exposure to trichloroethene in vivo and its metabolite S-(dichlorovinyl)-L-cysteine in vitro Toxicology, 224(1-2,1-2), 108-118					
Data Type:	Mutation analysis for DCVC					
HERO ID:	700373					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as S-(dichlorovinyl)-L-cysteine (DCVC).
	Metric 2:	Test Substance Source	High	× 1	1	The study indicated that chemicals were purchased from Sigma-Aldrich unless otherwise specified (there was no other indication for DCVC).
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of DCVC was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	The study did not report using an appropriate negative control (i.e., Tsc-2 genotype and VHL gene mutation not evaluated in untreated cells).
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type. The ability to detect the VHL mutation was demonstrated in another cell line (A438 cells).
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were reported and appeared adequate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation (but not storage) for the transformation assay was reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Information on exposure administration was reported and consistency of administration is inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported (24 hours). The study authors provided a rationale for an extended exposure time relative to MNNG (i.e., time required for bioactivation of DCVC).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The study indicated that 10 uM DCVC was used for the cell transformation assay (i.e., a single dose). The dose was presumably selected based on cytotoxicity.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type. A metabolite of TCE (DCVC) was used directly.
Domain 4: Test Model						

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Study Citation: A. Mally, C. Walker, J. Everitt, W. Dekant, S. Vamvakas (2006). Analysis of renal cell transformation following exposure to trichloroethene in vivo and its metabolite S-(dichlorovinyl)-L-cysteine in vitro Toxicology, 224(1-2,1-2), 108-118
Data Type: Mutation analysis for DCVC
HERO ID: 700373

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Low	× 2	6	The test model was described but is not routinely used for the outcome of interest. This particular cell line (carrying the Tsc-2 germline mutation) is uniquely susceptible to cell transformation.
	Metric 15:	Number per Group	High	× 1	1	Based on data shown in Table 3, it appears that at least 7 experiments were performed. In each experiment, cells were seeded at 10,000 cells/60 mm dish (20-30 dishes per group).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology addressed the intended outcomes of interest (loss of heterozygosity [LOH] at the the TSC-2 locus and mutations in the VHL gene).
	Metric 17:	Consistency of Outcome Assessment	Unacceptable	× 1	4	The outcome was assessed in only 9 (of about 17) transformants that resulted from DCVC treatment.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in test design or procedures were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables unrelated to treatment were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical evaluations were described in the methods, but were not applied/appropriate to the mutation data.
	Metric 23:	Data Interpretation	High	× 2	2	Criteria for data evaluation were reported and appropriate for the study (genotype for Tsc-2 locus; presence or absence of mutation in exon 1-3 for VHL).
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	With respect to the transformation assay, relative survival was determined as (CFE treated dishes)/(CFE control dishes). Relative survival values were reported for each experiment in Table 3.
	Metric 25:	Reporting of Data	High	× 2	2	Data are reported for each transformed cell line.
Overall Quality Determination‡			Unacceptable⋆⋆		1.8	
Extracted			No			

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Study Citation:	A. Mally, C. Walker, J. Everitt, W. Dekant, S. Vamvakas (2006). Analysis of renal cell transformation following exposure to trichloroethene in vivo and its metabolite S-(dichlorovinyl)-L-cysteine in vitro Toxicology, 224(1-2,1-2), 108-118
Data Type:	Mutation analysis for DCVC
HERO ID:	700373

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 134: **In vitro evaluation results of Beland 1999 study on bacterial reverse mutation**

Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Bacterial reverse mutation for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as chloral hydrate. In the NTP report, a CASRN, structure, and chemical formula were provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported (including lot number).
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be 99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative (solvent-only) control groups were included.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were tested concurrently with each test substance. The identity of each positive control was reported and appropriate for different strains with and without metabolic activation. Positive controls yielded positive results.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described in detail and were applicable to the study type. This evaluation form was completed with respect to Haworth et al. 1983 (HERO ID 28947), which was cited in Table E1 of Beland 1999 to contain the detailed protocol for the bacterial reverse mutation assay.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported. Test substance storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration for the pre-incubation protocol was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The maximum dose was chosen based on solubility limits or cytotoxicity. The number of exposure groups was reported (at least 5 plus controls) and spacing was appropriate (100, 333, 1000, 3333, 4000, 5000, 6667, 7500, and/or 10000 µg/plate).

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Bacterial reverse mutation for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	High	× 1	1	The source and method of preparation of the rat liver S9 fraction was reported; the concentration of S9 in the bacterial mutagenicity assay was specified in the data table (10%).
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity and donor source of the bacterial strains used here were identified, and these strains are routinely used for the outcome of interest. It was noted in Haworth et al. (1983) that the cultures were "routinely checked for genetic integrity as recommended by Ames et al. (1975)."
	Metric 15:	Number per Group	High	× 1	1	Each assay was plated in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analysis not required by study type. However, raw data were provided and could be analyzed independently.
	Metric 23:	Data Interpretation	High	× 2	2	The criteria for a positive (as well and negative and equivocal) response were reported. A response was considered positive if a reproducible, dose-related increase in revertant colonies was observed (no minimum fold-increase required).
	Metric 24:	Cytotoxicity Data	High	× 1	1	According to Haworth et al. (1983), a dose-setting experiment was conducted to assess cytotoxicity (viability based on reduced numbers of colonies). Doses for the mutagenicity assay were selected so that the highest dose exhibited some degree of toxicity.

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Bacterial reverse mutation for chloral hydrate
HERO ID:	701161

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	All data are adequately reported.
Overall Quality Determination‡		High		1.2	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 135: **In vitro evaluation results of Beland 1999 study on sister chromatid exchange**

Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Sister chromatid exchange for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as chloral hydrate. The NTP report also provided a CASRN, structure, and chemical formula.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported (including lot number).
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be 99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative (DMSO-only) control groups were included.
	Metric 5:	Positive Controls	High	× 2	2	Appropriate concurrent positive controls were included (cyclophosphamide with activation and mitomycin C without activation).
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described in detail and were applicable to the study type. This evaluation form was completed with respect to Galloway et al. 1987 (HERO ID 7768), which was cited in Table E2 of Beland 1999 to contain the detailed protocol for sister chromatid exchanges.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported. Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration for the SCE assay protocol was reported and appropriate. It was reported that some treatment groups with higher doses of chloral hydrate (both with and without activation) were incubated an additional 8.8 hours to "maximize the number of second-division metaphase cells available for analysis" owing to cell cycle delay; this is considered appropriate given the study design.

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Sister chromatid exchange for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The maximum dose was chosen based on cytotoxicity. The number of exposure groups (at least 3 plus controls) and dose spacing was reported and appropriate for this assay.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The source and method of preparation of the rat liver S9 fraction was reported; however, the concentration of S9 in the bacterial mutagenicity assay was not specified.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Chinese hamster ovary cells were utilized, but no additional details were provided. This cell line is routinely used for the outcome of interest.
	Metric 15:	Number per Group	Medium	× 1	2	It appears that single cultures were used; this is acceptable for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	It is inferred from the text that the outcome was assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	50 second-division metaphase cells were scored per dose level (adequate based on recommendations for this study type).
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding was reported.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	A linear regression test of SCEs per chromosome vs the log of the dose was conducted. For individual doses, absolute increases in SCEs per chromosome of 20% or more over the solvent control were considered positive. It was stated that this 20% threshold corresponded to a probability of occurring by chance of less than 1% ($p < 0.01$). This threshold and data analysis was considered to be appropriate for the study design.

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Sister chromatid exchange for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	High	× 2	2	The study indicated that a positive response was a 20% or greater increase in SCEs over solvent controls and/or based on statistical analyses.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Methods for dose selection based on cytotoxicity were described in detail in Galloway et al. 1987 (HERO ID 7768).
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 136: **In vitro evaluation results of Beland 1999 study on bacterial reverse mutation**

Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Bacterial reverse mutation for TCE metabolites
HERO ID:	701161

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE metabolites were clearly identified by name (chloral hydrate, trichloroacetic acid, trichloroethanol).
	Metric 2:	Test Substance Source	Low	× 1	3	The commercial source of the test substances was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Negative controls were included based on Figure D12, but further details were not provided.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not reported to be included in the study design. However, positive results were obtained; therefore, this demonstrates the ability of the lab to detect a positive result.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were briefly described and cited to other references (Maron and Ames 1983), but appeared appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Test substance preparation and/or vehicle was not reported. Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposure administration was inferred to be consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported (and can be estimated from Figure D12).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration for the pre-incubation protocol was reported and appropriate. The exposure duration for the direct plate incorporation method was not reported, but assumed to be appropriate considering the citation for the protocol (Maron and Ames 1983).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups was reported (at least 4 plus controls) and appropriate for this assay.

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Bacterial reverse mutation for TCE metabolites
HERO ID:	701161

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Medium	× 1	2	The source and method of preparation of the rat liver S9 fraction was reported; however, the concentration of S9 in the bacterial mutagenicity assay was not specified (assumed to be appropriate based on cited publication).
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The identity of the S. typhimurium strain TA 104 was identified. No further details were provided. This strain is routinely used for the outcome of interest.
	Metric 15:	Number per Group	Low	× 1	3	The number of plates per treatment group was not reported. It is likely that one plate per treatment group was utilized, as there are no error bars on the graph in Figure D12. This is considered acceptable for the bacterial reverse mutation assay.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analysis was not conducted and standard deviations were not reported, so independent statistical analysis is not possible. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay.
	Metric 23:	Data Interpretation	Low	× 2	6	Evaluation criteria were not explicitly specified.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	It is not apparent that cytotoxicity was assessed or considered in the study design or interpretation of results (but not strictly required by study type).

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Bacterial reverse mutation for TCE metabolites
HERO ID:	701161

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	Data were reported by exposure group (Figure D12).
Overall Quality Determination‡		Unacceptable⋆⋆		2.1	
Extracted		No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 137: **In vitro evaluation results of Beland 1999 study on chromosomal aberrations**

Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Chromosomal aberrations for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as chloral hydrate. In the NTP report, a CASRN, structure, and chemical formula were provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported (including lot number).
	Metric 3:	Test Substance Purity		× 1	NA	The test substance was reported to be 99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative (DMSO-only) control groups were included.
	Metric 5:	Positive Controls	High	× 2	2	Appropriate concurrent positive controls were included (cyclophosphamide with activation and mitomycin C without activation).
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described in detail and were applicable to the study type. This evaluation form was completed with respect to Galloway et al. 1987 (HERO ID 7768), which was cited in Table E3 of Beland 1999 to contain the detailed protocol for chromosomal aberrations.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported. Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration for the SCE assay protocol was reported and appropriate. It was reported that some treatment groups were incubated for additional time after the exposure concluded because "cell cycle delay was anticipated in the absence of S9"; this is considered appropriate given the study design.

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Chromosomal aberrations for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The maximum dose was chosen based on cytotoxicity. The number of exposure groups (at least 3 plus controls) and dose spacing was reported and appropriate for this assay.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The source and method of preparation of the rat liver S9 fraction was reported; however, the concentration of S9 in the bacterial mutagenicity assay was not specified.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Chinese hamster ovary cells were utilized, but no additional details were provided. This cell line is routinely used for the outcome of interest.
	Metric 15:	Number per Group	Medium	× 1	2	It appears that single cultures were used; this is acceptable for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	It is inferred from the text that the outcome was assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	Low	× 2	6	100 first-division metaphase cells were scored at most dose levels. It was noted that "occasionally, when a high percentage of aberrant cells was present in the culture, fewer cells were scored." Scoring fewer cells due to high incidence of CAs in itself is not expected to have impacted results. However, 100 metaphases per treatment group is lower than recommended by study type.
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding was reported.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Chromosomal aberrations for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Medium	× 1	2	According to Galloway et al. 1987 (HEROID 7768), linear regression test of the percentage of cells with aberrations vs the log of the dose was used as a test for trend. For individual doses, absolute increases in CAs were evaluated with a statistical test described by Margolin et al. 1983 (pg 714-715) for mutagenicity data with binomial responses.
	Metric 23:	Data Interpretation	High	× 2	2	The criteria for a positive (and equivocal) response was clearly reported. A result was considered weakly positive based on a statistically significant difference for one dose point and a significant trend; significant differences for two or more doses was positive.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Methods for dose selection based on cytotoxicity were described in detail in Galloway et al. 1987 (HERO ID 7768).
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 138: **In vitro evaluation results of Beland 1999 study on a Mammalian tk and hprt mutation assay**

Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Mammalian tk and hprt mutation assay for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as chloral hydrate. In the NTP report, a CASRN, structure, and chemical formula were provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported (including lot number).
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be 99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Appropriate concurrent negative control groups were included, indicated by "0" dose on Figure D11. No details regarding the identity (i.e. vehicle or untreated; if vehicle, the identity of the vehicle) were provided.
	Metric 5:	Positive Controls	Not Rated	NA	NA	It is standard to include a positive control for the hprt and tk mammalian gene mutation assay. A positive control was not included, but positive results were obtained; therefore, this demonstrates the ability of the lab to detect a positive result.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were described very briefly and cited to other references, but appeared appropriate for the endpoint of interest.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported (CH dissolved in distilled water for the cytotoxicity assay; cells from the cytotoxicity assay plated to evaluate gene mutation). Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposure administration appeared consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported (can be estimated from Figure D11).

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Mammalian tk and hprt mutation assay for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	The study indicated that cells were plated from the cytotoxicity assay 3 days (tk locus) or 6-7 days (hprt locus) after the exposure period were reported. Based on information from the cytotoxicity assay, exposure was for 28 hours.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups (at least 3 plus controls) and dose spacing was reported and appropriate. Doses induced cytotoxicity, but it was not clearly stated how the doses were selected.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	It does not appear that metabolic activation was utilized, but not required. H2E1 V2 human lymphoblastoid cells expressing cytochrome P4502E1 were utilized.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	H2E1 V2 human lymphoblastoid cells expressing cytochrome P4502E1 were utilized. Few additional details were provided. The specific strain of human lymphoblastoid cells does not appear to be routinely utilized for the outcome of interest; however, another strain of human lymphoblastoid cells are, so this is considered acceptable.
	Metric 15:	Number per Group	High	× 1	1	The briefly described methods indicates that triplicate cultures and duplicate experiments were used to assess the mutation frequency at each locus.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	It is inferred from the text that the outcome was assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Mammalian tk and hprt mutation assay for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical methods were not described (not strictly required by study type).
	Metric 23:	Data Interpretation	Medium	× 2	4	The study did not explicitly specify the criteria for a positive result. However, the results section described fold-changes and dose-dependency of effects.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Relative survival (%) was determined, but methods were not described in detail.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡			Medium		1.8	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 139: **In vitro evaluation results for Crebelli et al 1991 for fungal chromosome segregation study**

Study Citation:	R. Crebelli, G. Conti, L. Conti, A. Carere (1991). In vitro studies with nine known or suspected spindle poisons: results in tests for chromosome malsegregation in Aspergillus nidulans Mutagenesis, 6(2,2), 131-136
Data Type:	Chromosome segregation in Aspergillus nidulans for CH
HERO ID:	701624

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	CH was identified by established nomenclature.
	Metric 2:	Test Substance Source	Low	× 1	3	The test substance was provided by Professor J.M.Parry, University College of Swansea, Swansea, UK. Analytical verification of the test substance was not reported. It is unclear if the original source was a manufacturer.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Study authors reported using a concurrent negative control group, but all conditions were not equal to those of treated groups. Untreated controls were used instead of vehicle (distilled water) controls.
	Metric 5:	Positive Controls	High	× 2	2	A positive control (benomyl) was used and a positive response was observed.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and were cited in another publication, but appeared to be appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation in distilled water was described. Storage was not described (but not expected to impact results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	Exposure duration was reported (3 to 4.5 hours, until emergence of germ tubes) and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Concentrations were not justified, but the number of exposure groups and spacing of exposure levels were adequate to show results relevant to the outcome of interest. Only one dose was used in the haploid strain (no justification was provided).

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Study Citation:	R. Crebelli, G. Conti, L. Conti, A. Carere (1991). In vitro studies with nine known or suspected spindle poisons: results in tests for chromosome malsegregation in Aspergillus nidulans Mutagenesis, 6(2,2), 131-136
Data Type:	Chromosome segregation in Aspergillus nidulans for CH
HERO ID:	701624

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported along with limited descriptive information. The test model was routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	The number of colonies scored per group was reported and appropriate for the study type and outcome.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methods were reported and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups .
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no differences reported for initial study group parameters.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	There were no reported differences among the study groups unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	Statistical analysis was performed but the methods were not described clearly. Data were presented as number/percent whole chromosome or cross-overs in abnormal and normal colonies.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were not clearly reported. However, statistical significance was one of the criteria for a positive response.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were defined, but the methods of measurements were not fully described or reported.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.

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Study Citation:	R. Crebelli, G. Conti, L. Conti, A. Carere (1991). In vitro studies with nine known or suspected spindle poisons: results in tests for chromosome malsegregation in Aspergillus nidulans Mutagenesis, 6(2,2), 131-136
Data Type:	Chromosome segregation in Aspergillus nidulans for CH
HERO ID:	701624

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		Medium		1.7	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 140: **In vitro evaluation results for Gibson et al 1995 for mammalian cell transformation study**

Study Citation:	D. Gibson, M. Aardema, G. Kerckaert, G. Carr, R. Brauninger, R. LeBoeuf (1995). Detection of aneuploidy-inducing carcinogens in the Syrian hamster embryo (SHE) cell transformation assay Mutation Research, 343(1,1), 7-24
Data Type:	Mammalian transformation assay for chloral hydrate
HERO ID:	702114

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as chloral hydrate. A CASRN was also provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported. Although a batch/lot number was not reported, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included.
	Metric 5:	Positive Controls	High	× 2	2	A concurrent positive control (benzo[a]pyrene) was included. The positive control yielded positive results.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were briefly described (modifications) and cited to another publication (LeBoeuf et al. 1989).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported. Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (24 hours or 7 days) and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups was reported (5 or 6 groups plus controls) and appropriate for this assay. Dose selection was based on cytotoxicity considerations.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 4: Test Model						

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Study Citation:	D. Gibson, M. Aardema, G. Kerckaert, G. Carr, R. Brauninger, R. LeBoeuf (1995). Detection of aneuploidy-inducing carcinogens in the Syrian hamster embryo (SHE) cell transformation assay Mutation Research, 343(1,1), 7-24
Data Type:	Mammalian transformation assay for chloral hydrate
HERO ID:	702114

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	The test model (Syrian hamster embryo cells) was reported with limited information. The study indicated that frozen isolates of primary embryo cells were prepared from 12 day gestation hamsters (obtained from a commercial source); cells were used at passage 1 or 2.
	Metric 15:	Number per Group	Medium	× 1	2	The number of technical replicates for each experimental condition was not explicitly specified; however, the study was designed to evaluate sufficient numbers of colonies to detect a toxicological effect.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	About 1000 colonies per experimental group were evaluated.
	Metric 19:	Blinding of Assessors	High	× 1	1	It was reported that all plates, including positive and negative controls, were coded, mixed, and scored blind.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Data were appropriately analyzed by one-sided Fisher's exact test. A result was considered positive if two doses were significant, or if one dose was significant with a significant trend test.
	Metric 23:	Data Interpretation	High	× 2	2	The study used statistical significance as a criteria for a positive response. A chemical was concluded to be positive if it induced a significant increase morphological transformation frequency in at least two doses or at one dose with a significant trend test.
	Metric 24:	Cytotoxicity Data	High	× 1	1	A preliminary toxicity assay was conducted to assess cytotoxicity levels. The doses for the mutagenicity assay were selected so that approximately 50% survival was permitted, as determined by relative plating efficiency.

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Study Citation:	D. Gibson, M. Aardema, G. Kerckaert, G. Carr, R. Brauninger, R. LeBoeuf (1995). Detection of aneuploidy-inducing carcinogens in the Syrian hamster embryo (SHE) cell transformation assay Mutation Research, 343(1,1), 7-24
Data Type:	Mammalian transformation assay for chloral hydrate
HERO ID:	702114

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	Data were reported adequately.
Overall Quality Determination‡		High		1.3	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 141: **In vitro evaluation results for Giller et al 1995 for Ames fluctuation test study**

Study Citation:	S. Giller, F. Le Curieux, L. Gauthier, F. Erb, D. Marzin (1995). Genotoxicity assay of chloral hydrate and chloropicrine Mutation Research, 348(4,4), 147-152
Data Type:	in vitro SOS chromotest and ames fluctuation test Chloral hydrate
HERO ID:	702123

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chloral hydrate identified by name, molecular formula, and CASRN (CI3CCH(OH)2, CAS 302-17-0)
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source reported: Aldrich, (St Quentin Falavier, France)
	Metric 3:	Test Substance Purity	High	× 1	1	99% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent control was reported, but it is unclear if it is solvent or untreated
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive control was reported, but positive results were reported for CH and another tested compound
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Methods were cited to prior publications
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage were not reported
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Methods were cited to prior publications
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported unambiguously (ug/mL)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported (2 h for SOS chromotest and 72 h for Ames fluctuation) and adequate for the study type
	Metric 12:	Exposure Route and Method	High	× 1	1	Number and spacing of exposure concentrations were reported and appeared appropriate (7 concentrations plus control; overall range 3 orders of magnitude). Highest concentrations were toxic.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Metabolic activation was reported (S9 fraction from Aroclor 1254-induced Sprague Dawley rats) and commonly used, however details of administration were not described
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test models (S. typhimurium Strain TA 100 and E. coli) were reported and is routinely used for the outcomes.

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Study Citation:	S. Giller, F. Le Curieux, L. Gauthier, F. Erb, D. Marzin (1995). Genotoxicity assay of chloral hydrate and chloropicrine Mutation Research, 348(4,4), 147-152
Data Type:	in vitro SOS chromotest and ames fluctuation test Chloral hydrate
HERO ID:	702123

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	2 independent assays of 6 replicates/dose (SOS) or 3 replicates/dose (Ames).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Outcome assessment was cited to another publication without additional details
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Outcome assessment was cited to another publication without additional details
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the study type. Outcome assessment was cited to another publication without additional details
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial batch/lot number of organisms or models used per group, size, and/or quality of tissues exposed was not reported
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analysis not necessarily required for these outcomes. Statistical methods reported for Ames assay.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria were briefly described and were consistent with established practice
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity was assessed (based on toxicity noted in table of results), but the methods of measurements were not fully described or reported.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all outcomes by group in table 1
Overall Quality Determination‡			Unacceptable⋆⋆		1.6	
Extracted			Yes			

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Study Citation:	S. Giller, F. Le Curieux, L. Gauthier, F. Erb, D. Marzin (1995). Genotoxicity assay of chloral hydrate and chloropicrine Mutation Research, 348(4,4), 147-152
Data Type:	in vitro SOS chromotest and ames fluctuation test Chloral hydrate
HERO ID:	702123

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 142: **In vitro evaluation results for Jaffe et al 1985 for perfused kidney and isolated tubule alkaline elution assay study**

Study Citation:	D. Jaffe, C. Hassall, A. Gandolfi, K. Brendel (1985). Production of DNA single strand breaks in rabbit renal tissue after exposure to 1,2-dichlorovinylcysteine Toxicology, 35(1,1), 25-33
Data Type:	DCVC Studies perfused kidney and isolated tubules alkaline elution assay
HERO ID:	704496

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	1,2-dichlorovinylcysteine (DCVC) identified by name
	Metric 2:	Test Substance Source	Medium	× 1	2	DCVC was synthesized; methods cited to another publication
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Untreated negative controls were reported
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive controls were used and were not applicable for the study type
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods were described and appropriate for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported. Storage conditions were not reported but are not likely to significantly impact results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported in mM (fig 2 and 3)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure durations were reported in text (45 min for perfused kidney, 30 mi nfor renal tubules) and appeared to be appropriate for the study type
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Number of exposure groups as indicated by figures is control plus 3 treated groups. Concentration spacing appeared appropriate (gave a range of responses), but justification was not reported
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable for the study type
Domain 4: Test Model						

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Study Citation:	D. Jaffe, C. Hassall, A. Gandolfi, K. Brendel (1985). Production of DNA single strand breaks in rabbit renal tissue after exposure to 1,2-dichlorovinylcysteine Toxicology, 35(1,1), 25-33
Data Type:	DCVC Studies perfused kidney and isolated tubules alkaline elution assay
HERO ID:	704496

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	The animals from which the renal tissues were taken were clearly reported and purchased from a commercial source . Methods for isolating renal tubules were cited to another publication. The models (ex vivo kidney and isolated primary tubule cells) are appropriate for the outcome of interest.
	Metric 15:	Number per Group	Medium	× 1	2	At least 3 per group, not specified further. Adequate for the study type
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (alkaline elution) was fully described and appropriate for this endpoint.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome protocol was consistent across study groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial size and/or quality of tissues exposed was not reported. These
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was reported as one way ANOVA and Newman-Kuels test and was appropriate for the data set
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were briefly described and appeared appropriate for the study
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Not applicable to the study type
	Metric 25:	Reporting of Data	Medium	× 2	4	Data were reported graphically by exposure group, including mean and standard deviation for rate constant. n/group not reported.
Overall Quality Determination‡			Medium		1.7	
Extracted			Yes			

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Study Citation:	D. Jaffe, C. Hassall, A. Gandolfi, K. Brendel (1985). Production of DNA single strand breaks in rabbit renal tissue after exposure to 1,2-dichlorovinylcysteine Toxicology, 35(1,1), 25-33
Data Type:	DCVC Studies perfused kidney and isolated tubules alkaline elution assay
HERO ID:	704496

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 143: **In vitro evaluation results for Leuschner and Leuschner 1991 for bacterial reverse mutation study**

Study Citation:	J. Leuschner, F. Leuschner (1991). Evaluation of the mutagenicity of chloral hydrate in vitro and in vivo Arzneimittel-Forschung, 41(10,10), 1101-1103					
Data Type:	Bacterial reverse mutation, chloral hydrate					
HERO ID:	706734					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chloral hydrate was identified by name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source was identified. Batch/lot number was not provided, but the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	>99.4%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Solvent control was used.
	Metric 5:	Positive Controls	High	× 2	2	Several positive controls were used; text indicated that positive controls responded as expected.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and cited in another publication, but appeared to be appropriate
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance was prepared in aqueous vehicle. Storage conditions were not described, but this is not likely to have a substantial effect on the results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (ug/plate).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	48h incubation
	Metric 12:	Exposure Route and Method	Medium	× 1	2	5 doses up to 5000 ug/plate were used. The doses and spacing were not justified, and no information on cytotoxicity was provided.
	Metric 13:	Metabolic Activation	High	× 1	1	Aroclor 1254-induced rat liver S9; 500 ul 10% S9 used
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model (S. typhimurium, 5 strains) was reported but source was not identified.
	Metric 15:	Number per Group	High	× 1	1	3 plates/concentration, 2 independent experiments
Domain 5: Outcome Assessment						

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Study Citation:	J. Leuschner, F. Leuschner (1991). Evaluation of the mutagenicity of chloral hydrate in vitro and in vivo Arzneimittel-Forschung, 41(10,10), 1101-1103
Data Type:	Bacterial reverse mutation, chloral hydrate
HERO ID:	706734

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The method was sensitive for the outcome of interest. 5 Salmonella strains
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable for the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable for the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial batch/lot and number of organisms were not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analysis was not described but is not necessarily required for Ames assay
	Metric 23:	Data Interpretation	High	× 2	2	Criteria for a positive finding was reported and appropriate (dose-related >2-fold increase in revertants compared to the solvent control).
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.
	Metric 25:	Reporting of Data	Unacceptable	× 2	8	Text indicated that findings were negative and positive controls responded appropriately; however, no data were provided.
Overall Quality Determination‡			Unacceptable⋆⋆		1.6	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 144: **In vitro evaluation results for Lynch and Parry 1993 for micronucleus study**

Study Citation:	A. Lynch, J. Parry (1993). The cytochalasin-B micronucleus/kinetochore assay in vitro: studies with 10 suspected aneugens Mutation Research, 287(1,1), 71-86
Data Type:	in vitro micronucleus assay in Chinese hamster lung fibroblasts - CH
HERO ID:	706842

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name as chloral hydrate
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported as a commercial source (Sigma). The product number and batch/lot number were also not reported; however, the material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors report using concurrent untreated and solvent controls.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were used (colchicine and vinblastine).
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described in detail and applicable to the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Test substance preparation and storage conditions for CH were not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across treated and control groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentrations were reported without ambiguity (ug/ml)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (24 hours) and appropriate
	Metric 12:	Exposure Route and Method	High	× 1	1	A suitable concentration range was determined in an initial assay. 8 concentrations (up to a toxic concentration of 1600 ug/mL) were tested.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable for this study (metabolite tested)
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (low passage Chinese hamster cell line Luc2) and source were described and appropriate for the outcome of interest.

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Study Citation:	A. Lynch, J. Parry (1993). The cytochalasin-B micronucleus/kinetochore assay in vitro: studies with 10 suspected aneugens Mutation Research, 287(1,1), 71-86
Data Type:	in vitro micronucleus assay in Chinese hamster lung fibroblasts - CH
HERO ID:	706842

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number cells per study group were reported and were appropriate for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were described in detail and appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across the controls and treated groups.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	There were small differences in the numbers of cells scored per treatment group but these are unlikely to impact the results.
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Authors report using coded slides in calibration assay and it is assumed (but not specified) that the same method was used in the main assay.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were described and appropriate for the dataset.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria were reported and appropriate.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoints were defined and the concentration at which cytotoxicity occurred was reported.
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcome were presented for the control and treatment group for one set of hepatocytes; a second set of experiments was noted to have identical results (+/- 5%, but was not reported).
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	A. Lynch, J. Parry (1993). The cytochalasin-B micronucleus/kinetochore assay in vitro: studies with 10 suspected aneugens Mutation Research, 287(1,1), 71-86
Data Type:	in vitro micronucleus assay in Chinese hamster lung fibroblasts - CH
HERO ID:	706842

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 145: **In vitro evaluation results for Mcgregor et al 1989 for bacterial reverse mutation study**

Study Citation:	D. B. Mcgregor, D. M. Reynolds, E. Zeiger (1989). Conditions affecting the mutagenicity of trichloroethylene in Salmonella Environmental and Molecular Mutagenesis, 13(3,3), 197-202
Data Type:	bacterial reverse mutation
HERO ID:	706963

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by established nomenclature.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer was reported. Batch/lot number was not given, but the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade was not reported. Paper reports that substances were analyzed for purity at RTI.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	DMSO was used as the vehicle control for the preincubation assay. Control air was used for the vapor assay.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls (dichloromethane and 2-aminoanthracene) were used and expected responses were observed.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and cited to other publications. Methods appeared to be appropriate for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was described. Storage was not described, but the exposure period was only 48h.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were consistent across groups,
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported as ug/plate or % concentration in air.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appropriate for the study type (48h).
	Metric 12:	Exposure Route and Method	High	× 1	1	5-6 concentrations per assay. Highest concentrations produced toxicity.

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Study Citation:	D. B. Mcgregor, D. M. Reynolds, E. Zeiger (1989). Conditions affecting the mutagenicity of trichloroethylene in Salmonella Environmental and Molecular Mutagenesis, 13(3,3), 197-202
Data Type:	bacterial reverse mutation
HERO ID:	706963

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	High	× 1	1	Study authors reported exposures were conducted in the presence of metabolic activation and the type/source and volume in final culture were provided.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported along with limited descriptive information. The test model is routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	The number of replicates per study group was reported and appropriate (3 plates/concentration).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methods reported and were sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial number of cells per replicate was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences among the study replicates or groups in test model unrelated to exposure .
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	No statistical analyses were conducted but sufficient data were provided to conduct an independent statistical analysis.
	Metric 23:	Data Interpretation	Low	× 2	6	No evaluation criteria were provided.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Toxicity was judged to have occurred if there were reductions in colony number and/or clear reduction in microcolony number, as subjectively assessed with the aid of a low power microscope.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were reported quantitatively by exposure group (mean, SD, and n).
Overall Quality Determination‡			High		1.4	

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Study Citation:	D. B. Mcgregor, D. M. Reynolds, E. Zeiger (1989). Conditions affecting the mutagenicity of trichloroethylene in Salmonella Environmental and Molecular Mutagenesis, 13(3,3), 197-202
Data Type:	bacterial reverse mutation
HERO ID:	706963

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i \left(\text{Metric Score}_i \times \text{MWF}_i\right) / \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 146: **In vitro evaluation results for Natarajan et al 1993 for aneuploidy study in Chinese hamster embryonic cells**

Study Citation:	A. Natarajan, W. Duivenvoorden, M. Meijers, T. Zwanenburg (1993). Induction of mitotic aneuploidy using Chinese hamster primary embryonic cells. Test results of 10 chemicals Mutation Research, 287(1,1), 47-56
Data Type:	Aneuploidy for chloral hydrate
HERO ID:	707185

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name as chloral hydrate.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (DMSO and medium).
	Metric 5:	Positive Controls	High	× 2	2	Diethylstilbestrol was used as positive control and yielded expected results
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described adequately and appropriate to the outcome of interest.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported. Test substance storage was not reported but this is not expected to significantly impact the results (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (ug/ml).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (26 hr) and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (9 plus control) and dose spacing were appropriate.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The identity of the Chinese hamster embryo cells was reported. Other information regarding the cells was cited to other references.
	Metric 15:	Number per Group	Medium	× 1	2	The technical replicates per experimental condition were somewhat lacking (n = 2).
Domain 5: Outcome Assessment						

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Study Citation:	A. Natarajan, W. Duivenvoorden, M. Meijers, T. Zwanenburg (1993). Induction of mitotic aneuploidy using Chinese hamster primary embryonic cells. Test results of 10 chemicals Mutation Research, 287(1,1), 47-56
Data Type:	Aneuploidy for chloral hydrate
HERO ID:	707185

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology was partially described and appropriate for the outcome of interest. Some methods were cited to other publications.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	The sampling was somewhat lacking at 200 well-spread metaphases per experimental condition (100 cells per replicate).
	Metric 19:	Blinding of Assessors	High	× 1	1	It was reported that all slides were coded before analysis.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	No statistical analysis was conducted; however, raw data for chloral hydrate are presented in Table 3.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (observed numbers and numbers corrected for growth delay) was reported and appeared to be appropriate
	Metric 24:	Cytotoxicity Data	High	× 1	1	A pilot study to determine the range of doses to be tested was conducted. Depression of mitotic index was the measurement of cytotoxicity in the pilot study.
	Metric 25:	Reporting of Data	High	× 2	2	All data are reported adequately.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 147: **In vitro evaluation results for Ni et al 1994 for bacterial reverse mutation study**

Study Citation:	Y. Ni, T. Wong, F. Kadlubar, P. Fu (1994). Hepatic metabolism of chloral hydrate to free radical(s) and induction of lipid peroxidation Biochemical and Biophysical Research Communications, 204(2,2), 937-943
Data Type:	bacterial reverse mutation assay of chloral hydrate
HERO ID:	707204

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by name
	Metric 2:	Test Substance Source	High	× 1	1	Source reported as Sigma Chemical co (St Louis, MO)
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Figure 3 has a point at 0 mg/plate dose so it is inferred that a negative control was used. It is not clear if it is solvent or untreated.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not reported
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were cited to prior publications and briefly described and were applicable to the study type
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Limited details on preparation were provided; it is possible that details were provided in cited publications. Storage conditions were not reported but not expected to influence results of this short duration study.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Exposure methods were cited to another publication with no additional details.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses reported in figure legend as mg/plate
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	Exposure methods were cited to another publication with no additional details.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Number of exposure groups was control + 3. Spacing was not justified but appeared sufficient to induce a range of responses
	Metric 13:	Metabolic Activation	Medium	× 1	2	Metabolic activation was briefly described and a commonly used system.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model is commonly used, however only 2 strains were used (justified as sensitive to mutations by free radicals) and some details were not reported

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Study Citation:	Y. Ni, T. Wong, F. Kadlubar, P. Fu (1994). Hepatic metabolism of chloral hydrate to free radical(s) and induction of lipid peroxidation Biochemical and Biophysical Research Communications, 204(2,2), 937-943
Data Type:	bacterial reverse mutation assay of chloral hydrate
HERO ID:	707204

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	Tests were done in triplicate
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	The outcome assessment methodology was cited to another publication with no additional details
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	The outcome assessment methodology was cited to another publication with no additional details
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial batch/lot and number of organisms used per group was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported but this deficiency is unlikely to impact results
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical methods were not reported but not necessarily required for the outcome type
	Metric 23:	Data Interpretation	Low	× 2	6	Evaluation criteria were not reported.
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.
	Metric 25:	Reporting of Data	Medium	× 2	4	Means were reported graphically for all groups; no measure of variability reported
Overall Quality Determination‡			Unacceptable⋆⋆		2.1	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 148: **In vitro evaluation results for Robbiano et al 2004 for rat and human renal cell micronucleus study**

Study Citation:	L. Robbiano, D. Baroni, R. Carrozzino, E. Mereto, G. Brambilla (2004). DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney Toxicology, 204(2-3,2-3), 187-195
Data Type:	TCE induced MN in primary rat and human kidney cells
HERO ID:	707588

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was reported by name
	Metric 2:	Test Substance Source	High	× 1	1	The test substance source was reported as E. Merk (Darmstadt Germany), batch was not reported
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was reported as reagent grade
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	negative (solvent) controls were reported
	Metric 5:	Positive Controls	High	× 2	2	positive control (NDMA) was reported and appropriate
	Metric 6:	Assay Procedures	Medium	× 1	2	primary rat kidney isolation, human isolation method and comet assay were previously cited and briefly described
	Metric 7:	Standards for Tests	Not Rated	NA	NA	not applicable to the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	preparation of the test substance was reported from EtOH soln into cell media, storage was not described.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	previously cited methods did not describe
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	concentrations were reported in mM
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	exposure duration was reported (48h) and was appropriate for the study type
	Metric 12:	Exposure Route and Method	High	× 1	1	number of exposure groups was 3 + a positive and negative control and is adequate for the study type, high dose was determined from cytotoxicity assay and spacing appeared adequate to show dose response
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	not applicable to the study type
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	test model (rat and human primary kidney cells) was reported with limited description and appears appropriate for the outcome of interest

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Study Citation:	L. Robbiano, D. Baroni, R. Carrozzino, E. Mereto, G. Brambilla (2004). DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney Toxicology, 204(2-3,2-3), 187-195
Data Type:	TCE induced MN in primary rat and human kidney cells
HERO ID:	707588

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	methods were cited previously; cell count were not reported, but figure 1 reports analysis was done on independent experiments and cells were collected from 3 rats or human donors/concentration
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	outcome assessment methodology was previously cited but the brief description appeared a adequate for the outcome of interest
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	outcome assessment was inferred to be consistent across study groups according to previous citation and brief description
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	method was sited previously but sample number was not reported (MN)
	Metric 19:	Blinding of Assessors	Low	× 1	3	blinding was not reported (MN)
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	confounding variables in test design and procedures was not reported
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	confounding variables in outcomes unrelated to exposure weere not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	statistical analysis (MN) was reported as by the method of Bailey 1959 and data provided in figure 1 and table 1 are sufficient for independent analysis
	Metric 23:	Data Interpretation	Medium	× 2	4	criteria were assumed to be previously reported and appeared consistent with established practice (dose response, stat sig increase)
	Metric 24:	Cytotoxicity Data	High	× 1	1	concentrations used were chosen based on a cytotoxicity assay: highest dose had <30% reduction in cell viability
	Metric 25:	Reporting of Data	High	× 2	2	data were presented for all treated groups (as ratio) in fig 1 and positive and negative controls were identified in the fiure 1 legend
Overall Quality Determination‡			High		1.6	
Extracted			Yes			

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Study Citation:	L. Robbiano, D. Baroni, R. Carrozzino, E. Mereto, G. Brambilla (2004). DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney Toxicology, 204(2-3,2-3), 187-195
Data Type:	TCE induced MN in primary rat and human kidney cells
HERO ID:	707588

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 149: **In vitro evaluation results for Robbiano et al 2004 for human and rat renal cell DNA fragmentation study**

Study Citation:	L. Robbiano, D. Baroni, R. Carrozzino, E. Mereto, G. Brambilla (2004). DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney Toxicology, 204(2-3,2-3), 187-195
Data Type:	TCE induced DNA fragmentation in primary rat and human kidney cells
HERO ID:	707588

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was reported by name
	Metric 2:	Test Substance Source	High	× 1	1	The test substance source was reported as E. Merk (Darmstadt Germany), batch was not reported
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was reported as reagent grade
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	negative (solvent) controls were reported
	Metric 5:	Positive Controls	High	× 2	2	positive control (NDMA) was reported and appropriate
	Metric 6:	Assay Procedures	Medium	× 1	2	primary rat kidney isolation, human isolation method and comet assay were previously cited and briefly described
	Metric 7:	Standards for Tests	Not Rated	NA	NA	not applicable to the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	preparation of the test substance was reported from EtOH soln into cell media, storage was not described.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	previously cited methods did not describe
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	concentrations were reported in mM
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	exposure duration was reported (20h) exceeded the recommended 3-6 hours, but was considered acceptable for the study type
	Metric 12:	Exposure Route and Method	High	× 1	1	number of exposure groups was 3 + a positive and negative control and is adequate for the study type, high dose was determined from cytotoxicity assay and spacing appeared adequate to show dose response
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	not applicable to the study type
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	test model (rat and human primary kidney cells) was reported with limited description and appears appropriate for the outcome of interest

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Study Citation:	L. Robbiano, D. Baroni, R. Carrozzino, E. Mereto, G. Brambilla (2004). DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney Toxicology, 204(2-3,2-3), 187-195
Data Type:	TCE induced DNA fragmentation in primary rat and human kidney cells
HERO ID:	707588

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	methods were cited previously; cell count were not reported, but figure 1 reports analysis was done on independent experiments and cells were collected from 3 rats or human donors/concentration
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	outcome assessment methodology was previously cited but the brief description appeared a adequate for the outcome of interest
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	outcome assessment was inferred to be consistent across study groups according to previous citation and brief description
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	not applicable to the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	not applicable to the study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	confounding variables in test design and procedures was not reported
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	confounding variables in outcomes unrelated to exposure weere not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	data provided in figure 1 and figure 1 legend are sufficient for independent analysis
	Metric 23:	Data Interpretation	Medium	× 2	4	criteria were assumed to be previously reported and appeared consistent with established practice (dose response, stat sig increase)
	Metric 24:	Cytotoxicity Data	High	× 1	1	concentrations used were chosen based on a cytotoxicity assay: highest dose had <30% reduction in cell viability
	Metric 25:	Reporting of Data	High	× 2	2	data were presented for all treated groups (as ratio) in fig 1 and positive and negative controls were identified in the fiure 1 legend
Overall Quality Determination‡			High		1.6	
Extracted			Yes			

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Study Citation:	L. Robbiano, D. Baroni, R. Carrozzino, E. Mereto, G. Brambilla (2004). DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney Toxicology, 204(2-3,2-3), 187-195
Data Type:	TCE induced DNA fragmentation in primary rat and human kidney cells
HERO ID:	707588

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 150: **In vitro evaluation results for Sbrana et al 1993 for human lymphocyte chromosomal aberration study**

Study Citation:	I. Sbrana, A. Di Sibio, A. Lomi, V. Scarcelli (1993). C-mitosis and numerical chromosome aberration analyses in human lymphocytes: 10 known or suspected spindle poisons Mutation Research, 287(1,1), 57-70
Data Type:	in vitro chromosome aberration analyses in human lymphocytes - CH
HERO ID:	707750

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name as chloral hydrate
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported (J.M. Parry, Swansea, UK), but it was not a commercial manufacturer.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The results indicate the use of a control (0 ug/ml); however, it is unclear if it is an untreated or vehicle control)
	Metric 5:	Positive Controls	Not Rated	NA	NA	The use of a positive control was not reported. Other compounds tested were shown to induce positive results.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were described; however, some information was omitted (incubation temperature, humidity)
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was prepared by dissolving in distilled water; Information on storage was not reported but is not expected to significantly impact the results of this short duration study.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were reported to be administered consistently across treated and control groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentrations were reported without ambiguity (ug/ml)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (72 and 96 hours)
	Metric 12:	Exposure Route and Method	High	× 1	1	A suitable concentration range (4 to 8 doses plus control) was tested; doses were selected based on those producing spindle effects after long term treatments.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable for this study

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Study Citation:	I. Sbrana, A. Di Sibio, A. Lomi, V. Scarcelli (1993). C-mitosis and numerical chromosome aberration analyses in human lymphocytes: 10 known or suspected spindle poisons Mutation Research, 287(1,1), 57-70
Data Type:	in vitro chromosome aberration analyses in human lymphocytes - CH
HERO ID:	707750

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 4: Test Model					
Metric 14:	Test Model	High	× 2	2	The test model (human lymphocytes) and source were reported and appropriate for the outcome of interest.
Metric 15:	Number per Group	Medium	× 1	2	Two experiments performed per exposure condition
Domain 5: Outcome Assessment					
Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were described and appropriate for the endpoints of interest.
Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across the controls and treated groups.
Metric 18:	Sampling Adequacy	Medium	× 2	4	200 metaphases/dose evaluated; guidance recommends at least 300
Metric 19:	Blinding of Assessors	Low	× 1	3	Study did not report use of coded slides
Domain 6: Confounding / Variable Control					
Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial batch/lot number of organisms used per group was not reported.
Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported but this deficiency is not expected to influence results
Domain 7: Data Presentation and Analysis					
Metric 22:	Data Analysis	High	× 1	1	Statistical methods (FIsher's exact) were described and appropriate for the dataset
Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria were reported and appropriate.
Metric 24:	Cytotoxicity Data	High	× 1	1	Mitotic index evaluated as measure of cytotoxicity and results were reported for a range of doses.
Metric 25:	Reporting of Data	High	× 2	2	Results were presented for the control and all treatment groups.
Overall Quality Determination‡		High		1.6	
Extracted		No			

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Study Citation:	I. Sbrana, A. Di Sibio, A. Lomi, V. Scarcelli (1993). C-mitosis and numerical chromosome aberration analyses in human lymphocytes: 10 known or suspected spindle poisons Mutation Research, 287(1,1), 57-70
Data Type:	in vitro chromosome aberration analyses in human lymphocytes - CH
HERO ID:	707750

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 151: **In vitro evaluation results for Seelbach et al 1993 for micronucleus study**

Study Citation:	A. Seelbach, B. Fissler, S. Madle (1993). Further evaluation of a modified micronucleus assay with V79 cells for detection of aneugenic effects Mutation Research, 303(4,4), 163-169
Data Type:	in vitro modified micronucleus assay in V79 cells - CH
HERO ID:	707821

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate with CAS number.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported as a commercial source. The product number and batch/lot number were not reported; however, the material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Study authors report using a concurrent untreated negative control. Solvent for CH was water so solvent control not warranted.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were used (colcemid).
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were fully described and appeared appropriate
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance was prepared by dissolving in distilled water. The storage conditions for CH were not reported but are unlikely to affect the results of this short duration study
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across treated and control groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentration was reported without ambiguity in ug/ml
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (3 hours) and suited to the outcome
	Metric 12:	Exposure Route and Method	High	× 1	1	CH was tested up to the limit of solubility. The number (4 concentrations) and spacing (overall range 30x) of exposure concentrations were reported and appeared to be appropriate.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable for this study (metabolite tested)
Domain 4: Test Model						

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Study Citation:	A. Seelbach, B. Fissler, S. Madle (1993). Further evaluation of a modified micronucleus assay with V79 cells for detection of aneugenic effects Mutation Research, 303(4,4), 163-169
Data Type:	in vitro modified micronucleus assay in V79 cells - CH
HERO ID:	707821

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	The test model (V79 cells) was characterized. Private source was reported . Model is appropriate for the outcome of interest.
	Metric 15:	Number per Group	Medium	× 1	2	2 experiments per test condition
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were described appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across the controls and treated groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	Micronuclei were assessed in 2,000 cells group.
	Metric 19:	Blinding of Assessors	Low	× 1	3	Authors did not report using coded slides
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	Initial batch/lot number of organisms used per group was not reported
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported but this deficiency is not expected to impact the study results
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods (t-test) were described and appropriate for the dataset.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria were reported and appropriate ("MN frequency was reproducibly statistically significantly different from the control value")
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity was evaluated as mitotic index and reported for all tested concentrations.
	Metric 25:	Reporting of Data	High	× 2	2	Data were presented for the control and treatment groups for both experiments.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 152: **Animal toxicity evaluation results for Allen et al 1994 for short-term inhalation study on micronuclei in mouse spermatids**

Study Citation:	J. W. Allen, B. W. Collins, P. A. Evansky (1994). Spermatid micronucleus analyses of trichloroethylene and chloral hydrate effects in mice Mutation Research Letters, 323(1-2,1-2), 81-88
Data Type:	5-day inhalation Spermatid Micronucleus Assay in mice
HERO ID:	69053

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene (TCE); CAS number was reported.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified. The product number and batch/lot number was not reported; however the material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was reported (> 99 %)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were tested (conditioned air.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for this study.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation methodology was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation and storage conditions were reported and appropriate for the test substance . The method and equipment used to generate the test substance as a vapor were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups with consistent chamber designs, animals/chamber, and comparable characteristics in inhalation conditions.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentration were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for this endpoint (6 hours/day for 5 days)
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Though the study authors did not justify the number of exposure groups or concentration spacing, the number of exposure groups and spacing of exposure levels appear to be adequate to show results relevant to the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route was appropriate for the test substance.
Domain 4: Test Organism						

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Study Citation:	J. W. Allen, B. W. Collins, P. A. Evansky (1994). Spermatid micronucleus analyses of trichloroethylene and chloral hydrate effects in mice Mutation Research Letters, 323(1-2,1-2), 81-88
Data Type:	5-day inhalation Spermatid Micronucleus Assay in mice
HERO ID:	69053

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and age were reported while health status and starting body weight were not . The test animal was from a reported commercial source. The test species and strain were an appropriate animal model for the evaluation of this endpoint. The uncertainties in reporting are unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Some animal husbandry conditions were reported (light-dark cycle, diet, chamber conditions); however other conditions were not (temperature and humidity). Limitations are unlikely to have a substantial impact on results.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported, appropriate for the study type and outcome analysis (6 mice/group).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest (1,000 early round) spermatids from each animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for this study
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the negative control groups were adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not provided and TCE is considered to be a respiratory irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were described and were appropriate for the dataset (one-tailed trend test).
	Metric 24:	Reporting of Data	High	× 2	2	All data were adequately reported
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	J. W. Allen, B. W. Collins, P. A. Evansky (1994). Spermatid micronucleus analyses of trichloroethylene and chloral hydrate effects in mice Mutation Research Letters, 323(1-2,1-2), 81-88
Data Type:	5-day inhalation Spermatid Micronucleus Assay in mice
HERO ID:	69053

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 153: **In vitro evaluation results for Vagnarelli et al 1990 for human lymphocyte aneuploidy study**

Study Citation:	P. Vagnarelli, A. De Sario, L. De Carli (1990). Aneuploidy induced by chloral hydrate detected in human lymphocytes with the Y97 probe Mutagenesis, 5(6,6), 591-592
Data Type:	Aneuploidy in human lymphocytes CH
HERO ID:	708252

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Reported by name and CAS number
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source (Fluka A.G.) reported; batch number was not reported but test material not expected to vary in composition
	Metric 3:	Test Substance Purity	High	× 1	1	Purity reported 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	A negative control of 0 ug/ml was reported. It is unclear whether these are untreated or solvent controls. Vehicle was not reported for CH (reported for the positive control) so it is assumed that CH was administered in water.
	Metric 5:	Positive Controls	High	× 2	2	Benomyl was reported as a positive control
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods were partially described and cited to other publications but were appropriate for the study type
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Preparation of the test substance was briefly described, but lacked specific information on vehicle (assumed to be water). Storage was not reported but is unlikely to affect this short-duration study.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Consistent exposure administration was inferred from text, but details of the volume administered were not provided.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported in ug/ml without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported (24 hr) and appropriate to the endpoint.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups were 3 treated plus 2 controls (positive and negative). Doses were selected based on preliminary cytotoxicity studies and included both toxic and subtoxic doses.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable
Domain 4: Test Model						

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Study Citation:	P. Vagnarelli, A. De Sario, L. De Carli (1990). Aneuploidy induced by chloral hydrate detected in human lymphocytes with the Y97 probe Mutagenesis, 5(6,6), 591-592
Data Type:	Aneuploidy in human lymphocytes CH
HERO ID:	708252

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	Test model (PHA-stimulated peripheral blood lymphocytes from adult male donor) was described and appropriate for the study type
	Metric 15:	Number per Group	Low	× 1	3	Replicates were not performed (single experiment).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The outcome assessment methodology was partially described with reference to published methods. The description cited "standard protocols" for chromosome preparation without citation to a published method or guideline. The paper was validating a method (in situ hybridization on interphase nuclei with a chromosome Y-specific DNA probe).
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Outcome assessment was partially cited to other publications or "standard methods" but the uncertainties associated with these deficiencies are unlikely to significantly impact results.
	Metric 18:	Sampling Adequacy	High	× 2	2	1000-2000 nuclei were scored per dose group
	Metric 19:	Blinding of Assessors	Low	× 1	3	Authors did not report whether slides were coded prior to evaluation.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Number of cells/cell density per treatment group were not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Tetraploid cells were excluded from scoring; number excluded from each treatment group was not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis (G test) was described and data presented sufficiently to conduct independent analysis (mean, SE, and n)
	Metric 23:	Data Interpretation	High	× 2	2	Scoring criteria were reported and appropriate for the study type
	Metric 24:	Cytotoxicity Data	High	× 1	1	Study authors defined cytotoxicity endpoints (plating efficiency and mitotic index) and the methods for measuring cytotoxicity were clearly described and commonly used for assessment.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all groups and outcomes (mean, SE, and n)

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Study Citation:	P. Vagnarelli, A. De Sario, L. De Carli (1990). Aneuploidy induced by chloral hydrate detected in human lymphocytes with the Y97 probe Mutagenesis, 5(6,6), 591-592
Data Type:	Aneuploidy in human lymphocytes CH
HERO ID:	708252

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		High		1.6	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 154: **In vitro evaluation results for Vamvakas et al 1988 for bacterial reverse mutation study**

Study Citation:	S. Vamvakas, A. Elfarra, W. Dekant, D. Henschler, M. Anders (1988). Mutagenicity of amino acid and glutathione S-conjugates in the Ames test Mutation Research, 206(1,1), 83-90
Data Type:	Bacterial reverse mutation for DCVC, DCVG
HERO ID:	708267

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified by established nomenclature as S-(1,2-dichlorovinyl)-L-cysteine (DCVC) and S-1,2-dichlorovinyl)glutathione (DCVG).
	Metric 2:	Test Substance Source	High	× 1	1	The test substances were synthesized by the laboratory and verified by 1H-NMR and HPLC.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substances was determined to be at least 98.5% pure by 1H-NMR and HPLC.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Results for negative controls were shown graphically indicating that concurrent negative controls were used. It is unclear whether controls were vehicle (methanol) or untreated.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A concurrent positive control or proficiency group was not used but treatment-related positive responses were observed
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described in detail and were applicable to the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported (dissolved in methanol). Test substance storage was not reported but is unlikely to significantly impact results of this short duration study.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported in nmol in Figures 1 and 2.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (2 h) for the pre-incubation protocol was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (6 plus control) and dose spacing was sufficient to show a range of responses.
	Metric 13:	Metabolic Activation	High	× 1	1	The source and method of preparation of the rat kidney and liver isolates were reported.

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Study Citation: S. Vamvakas, A. Elfarra, W. Dekant, D. Henschler, M. Anders (1988). Mutagenicity of amino acid and glutathione S-conjugates in the Ames test Mutation Research, 206(1,1), 83-90
Data Type: Bacterial reverse mutation for DCVC, DCVG
HERO ID: 708267

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The identity of the bacterial strain was identified, but the source was not. It was reported that the properties of the strain, including UV and crystal violet sensitivity, ampicillin resistance, and UV mutability, were checked regularly.
	Metric 15:	Number per Group	High	× 1	1	Each assay was plated in duplicate, and each experiment was conducted 5 times.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported and is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to this endpoint. Colonies counted with automated counter.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	No statistical analysis was conducted, but is not necessarily required for bacterial reverse mutation . Means could be estimated from graphs, but no variance data was provided.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (doubling of colony number over control) was reported and consistent with current standards.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Based on notations in the text, cytotoxicity endpoints were considered, but the methods of measurement were not reported
	Metric 25:	Reporting of Data	Medium	× 2	4	Data were reported graphically for all treatment groups and controls. Mean and number of plates reported, but a measure of variance was not. Authors reported that "differences in colony counts from analogous plates did not exceed 20%".
Overall Quality Determination‡			High		1.4	

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Study Citation:	S. Vamvakas, A. Elfarra, W. Dekant, D. Henschler, M. Anders (1988). Mutagenicity of amino acid and glutathione S-conjugates in the Ames test Mutation Research, 206(1,1), 83-90
Data Type:	Bacterial reverse mutation for DCVC, DCVG
HERO ID:	708267

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 155: **In vitro evaluation results for Vamvakas et al 1996 for c-Fos expression study**

Study Citation:	S. Vamvakas, H. Richter, D. Bittner (1996). Induction of dedifferentiated clones of LLC-PK1 cells upon long-term exposure to dichlorovinylcysteine Toxicology, 106(1-3,1-3), 65-74					
Data Type:	c-Fos expression for DCVC					
HERO ID:	708268					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name (dichlorovinyl cysteine).
	Metric 2:	Test Substance Source	Medium	× 1	2	The details regarding synthesis of the test substance were incompletely reported, but omitted details are unlikely to impact the results. The study indicates that synthesis of the test substance was performed as described by Dekant et al. 1986 and Vamvakas et al. 1988. HPLC was used to analytically verify the identity of the test substance.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity and composition were such that any observed effects were highly likely to be due to the nominal test substance itself. HPLC analysis determined that the test substance was 99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using concurrent negative (untreated) control cells.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control is not required by study type. The test substance enhanced c-fos expression (indicating that the assay is capable of detecting a positive response).
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described in adequate detail (e.g., primary and secondary antibodies used to detect c-fos expression).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The timing of test substance preparation and test substance storage conditions were not explicitly reported; however, owing to the short duration of the study (up to 90 min) using the metabolite, the omitted information is not likely to significantly impact the study results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures appeared to be administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity (i.e., 1 or 5 uM).

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Study Citation:	S. Vamvakas, H. Richter, D. Bittner (1996). Induction of dedifferentiated clones of LLC-PK1 cells upon long-term exposure to dichlorovinylcysteine Toxicology, 106(1-3,1-3), 65-74
Data Type:	c-Fos expression for DCVC
HERO ID:	708268

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The duration of exposure (30, 60, or 90 minutes) was clearly reported, and appeared to be appropriate for the study type and outcome of interest.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	There were limitations regarding the number of exposure groups (2 + negative control); however, an exposure-response was observed for the outcome of interest. A rationale for the selection of these exposure concentrations was not provided (same as those used to evaluate dome formation).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation not required for this study type.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The cell type and descriptive information (tissue origin, number of passages, karyotype features) were reported, the test model was obtained from a named source (LLC-PK1 cells from American Type Culture Collection), and the test model was considered appropriate for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	The legend to Figure 7 indicates that the experiment was repeated three times.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no reported differences among study group parameters (e.g., test substance, cells used) that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables unrelated to exposure were identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Data were presented in Figure 7 as means +/- SD for 3 experiments.

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Study Citation:	S. Vamvakas, H. Richter, D. Bittner (1996). Induction of dedifferentiated clones of LLC-PK1 cells upon long-term exposure to dichlorovinylcysteine Toxicology, 106(1-3,1-3), 65-74
Data Type:	c-Fos expression for DCVC
HERO ID:	708268

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	Medium	× 2	4	The criteria for a positive response was not clearly specified (other than enhanced expression). However, the text mentions a 2-fold increase in expression in clones compared to controls.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 156: **In vitro evaluation results for Vamvakas et al 1996 for morphological transformation study**

Study Citation:	S. Vamvakas, H. Richter, D. Bittner (1996). Induction of dedifferentiated clones of LLC-PK1 cells upon long-term exposure to dichlorovinylcysteine Toxicology, 106(1-3,1-3), 65-74					
Data Type:	Morphological transformation, LLC-PK1 cells, DCVC					
HERO ID:	708268					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name (dichlorovinyl cysteine).
	Metric 2:	Test Substance Source	Medium	× 1	2	The details regarding synthesis of the test substance were incompletely reported, but omitted details are unlikely to impact the results. The study indicates that synthesis of the test substance was performed as described by Dekant et al. 1986 and Vamvakas et al. 1988. HPLC was used to analytically verify the identity of the test substance.
	Metric 3:	Test Substance Purity	High	× 1	1	HPLC analysis was used to determine that the test substance was 99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate concurrent negative control group (i.e., all conditions equal except chemical exposure).
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control not required for this study type.
	Metric 6:	Assay Procedures	Medium	× 1	2	The study authors described the methods and procedures (test conditions, culture medium and volumes, washing methods, instrument to quantify dome formation) used for the test and they appeared to be applicable for the study type. Temperature and humidity conditions, and initial cell density, were not reported.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable for this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	The test substance preparation was reported. It is not clear whether the cell medium was refreshed routinely over the 7 week exposure period, requiring additional test substance. The omitted information could significantly impact the study results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The exposure concentrations were reported without ambiguity (i.e., 1 or 5 uM DCVC).

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Study Citation:	S. Vamvakas, H. Richter, D. Bittner (1996). Induction of dedifferentiated clones of LLC-PK1 cells upon long-term exposure to dichlorovinylcysteine Toxicology, 106(1-3,1-3), 65-74
Data Type:	Morphological transformation, LLC-PK1 cells, DCVC
HERO ID:	708268

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (7 weeks) was reported and appeared to be appropriate for the study type and outcome of interest.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	There were limitations regarding the number of exposure groups (2 + negative control); however, an exposure-response was observed for the outcome of interest. A rationale for the selection of these exposure concentrations was not provided (except that "low, non-cytotoxic concentrations" were used).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable (metabolite tested)
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The cell type and descriptive information (tissue origin, number of passages, karyotype features) were reported, the test model was obtained from a named source (LLC-PK1 cells from American Type Culture Collection), and the test model was consisdered appropriate for the outcome of interest (i.e., chosen to investigate kidney cancer).
	Metric 15:	Number per Group	Low	× 1	3	The number of clones per exposure group were reported but considered low. Two clones with morphological alterations were derived from LLC-PK1 cells exposed to 1 uM DCVC (C1 and C2), and one clone was established from a monolayer exposed to 5 uM DCVC.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology reported the intended outcome of interest; minor uncertainties are unlikely to substantially impact the results. The study provided data for area of dome formation (with decreased dome area being considered an adverse effect); it was noted that DCVC-induced clones produced a higher number of domes than controls, and that controls and treated cells had the same proliferative capacity. Although it could be considered unclear why area was considered the appropriate assessment methodology (rather than numbers of domes, for example), subsequent biochemical experiments provided evidence that decreased dome area was associated with impaired apicobasolateral transport.

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Study Citation:	S. Vamvakas, H. Richter, D. Bittner (1996). Induction of dedifferentiated clones of LLC-PK1 cells upon long-term exposure to dichlorovinylcysteine Toxicology, 106(1-3,1-3), 65-74
Data Type:	Morphological transformation, LLC-PK1 cells, DCVC
HERO ID:	708268

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (days 5, 6, and 7 after seeding).
	Metric 18:	Sampling Adequacy	High	× 2	2	The number of evaluations per exposure group were considered adequate for the study type. To evaluate dome formation, 3 x 1 mm2 fields were analyzed. Data points represented 9 determinations from 3 separate experiments.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not mentioned in the study report; this metric is considered not applicable to this study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among study group parameters (e.g., test substance, cells used) that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences among the study replicates or groups in test model unrelated to exposure and the test substance did not interfere with the assay
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was briefly described. The legend for the figure containing data for area of dome formation indicated that data represented means and standard deviations from 9 determinations/3 experiments and that significance was determined by Student's t-test.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were briefly reported with some omissions (e.g., time points selected for analyses) and appeared to be appropriate for the study type.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	For the purposes of this study, low, non-cytotoxic concentrations were used (data were not shown but confluency was reported).
	Metric 25:	Reporting of Data	Medium	× 2	4	Data were provided for dome formation for each exposure group; other data (i.e., numbers of domes; not the focus of the outcome assessment) were reported qualitatively. However, the figure only provides data for area of dome formation expressed as % of controls; actual area data/measures of variation were not reported (but are not expected to substantially impact the results).

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Study Citation:	S. Vamvakas, H. Richter, D. Bittner (1996). Induction of dedifferentiated clones of LLC-PK1 cells upon long-term exposure to dichlorovinylcysteine Toxicology, 106(1-3,1-3), 65-74
Data Type:	Morphological transformation, LLC-PK1 cells, DCVC
HERO ID:	708268

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		High		1.5	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 157: **In vitro evaluation results for Warr et al 1993 for mammalian chromosome enumeration study**

Study Citation:	T. Warr, E. Parry, J. Parry (1993). A comparison of two in vitro mammalian cell cytogenetic assays for the detection of mitotic aneuploidy using 10 known or suspected aneugens Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 287(1,1), 29-46
Data Type:	Aneuploidy assay for CH (chromosome enumeration)
HERO ID:	708375

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The chemical (chloral hydrate) was clearly identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the chemical was reported (Sigma Chemical Company). Although a lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity or grade of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study reported using negative (untreated and solvent) controls in which the conditions were equal except for treatment.
	Metric 5:	Positive Controls	Medium	× 2	4	A concurrent positive control was used, but there were minor uncertainties. The study noted that suitable positive controls have not been well-established; however, a compound of known activity (2-acetylaminofluorene; 2-AAF) was included to confirm that conditions allowed induction of aberrations.
	Metric 6:	Assay Procedures	High	× 1	1	The study authors described the methods and procedures (e.g., test conditions, cell density, culture media, rinsing methods, slide preparation) used for the test in detail and they were applicable for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable for this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance preparation was reported. Storage conditions were not reported, but given the short-term duration of the study, this is not likely to substantially impact the study results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The exposure concentrations were reported in ug/ml without ambiguity.

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Study Citation:	T. Warr, E. Parry, J. Parry (1993). A comparison of two in vitro mammalian cell cytogenetic assays for the detection of mitotic aneuploidy using 10 known or suspected aneugens Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 287(1,1), 29-46
Data Type:	Aneuploidy assay for CH (chromosome enumeration)
HERO ID:	708375

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The duration of exposure was reported qualitatively (i.e., cells were exposed to the test substance for 1 cell cycle); this limitation us unlikely to have a substantial impact on the results.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	There were minor limitations regarding concentration spacing (no rationale provided), but the number of exposure groups (5 plus control) and spacing (15x overall range) of exposure levels were adequate to show results relevant to the outcome of interest (e.g., observation of a dose-response relationship) and the concerns are unlikely to have a substantial impact on results.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable for this study type (metabolite of TCE tested)
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported with limited descriptive information. Chinese hamster cell lines are routinely used for the outcome of interest. However, this particular pulmonary cell line (LUC2) is not routinely used. These limitations are unlikely to have a substantial impact on results.
	Metric 15:	Number per Group	High	× 1	1	The number of replicates per study group (n = 3) was reported and considered consistent with studies of the same or similar type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was fully described and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	120 to 300 cells/dose were scored across 3 replicates
	Metric 19:	Blinding of Assessors	High	× 1	1	Authors reported that slides were independently coded prior to scoring
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among study group parameters (e.g., test substance, cells used) that could influence the outcome assessment.

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Study Citation:	T. Warr, E. Parry, J. Parry (1993). A comparison of two in vitro mammalian cell cytogenetic assays for the detection of mitotic aneuploidy using 10 known or suspected aneugens Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 287(1,1), 29-46
Data Type:	Aneuploidy assay for CH (chromosome enumeration)
HERO ID:	708375

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported but this deficiency is not likely to significantly impact results
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were described ("control and treated cultures compared using Chi squared analysis and Fisher's exact test [for numbers below 10] at the 95% confidence limit").
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Criteria for chromosome number are not necessary.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity was noted with some results suggesting that the authors considered it, but the method of measurement was not reported.
	Metric 25:	Reporting of Data	Medium	× 2	4	Data for exposure-related findings were reported for outcomes by exposure group (e.g., percentages reported in the absence of mean data). The minor uncertainties in outcome reporting are unlikely to have substantial impact on results.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 158: **In vitro evaluation results for Warr et al 1993 for mammalian cell division study**

Study Citation:	T. Warr, E. Parry, J. Parry (1993). A comparison of two in vitro mammalian cell cytogenetic assays for the detection of mitotic aneuploidy using 10 known or suspected aneugens Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 287(1,1), 29-46
Data Type:	Aneuploidy assay for CH (cell division aberration)
HERO ID:	708375

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The chemical (chloral hydrate) was clearly identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the chemical was reported (Sigma Chemical Company). Although a lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity or grade of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study reported using negative (untreated and solvent) controls in which the conditions were equal except for treatment.
	Metric 5:	Positive Controls	Medium	× 2	4	A concurrent positive control or proficiency control was used, but there were minor uncertainties. The study noted that suitable positive controls for the assay have not been well-established; however, a compound of known activity (colcemid) was included to confirm that conditions allowed induction of aberrations.
	Metric 6:	Assay Procedures	High	× 1	1	The study authors described the methods and procedures (e.g., test conditions, cell density, culture media, rinsing methods, slide preparation) used for the test in detail and they were applicable for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable for this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance preparation was reported. Storage conditions were not reported, but given the short-term duration of the study, this is not likely to substantially impact the study results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The exposure concentrations were reported in ug/ml without ambiguity.

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Study Citation:	T. Warr, E. Parry, J. Parry (1993). A comparison of two in vitro mammalian cell cytogenetic assays for the detection of mitotic aneuploidy using 10 known or suspected aneugens Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 287(1,1), 29-46
Data Type:	Aneuploidy assay for CH (cell division aberration)
HERO ID:	708375

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The duration of exposure was reported qualitatively (i.e., cells were exposed to the test substance for 1 cell cycle); this limitation us unlikely to have a substantial impact on the results.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	There were minor limitations regarding concentration spacing (no rationale provided), but the number of exposure groups (4 plus control) and spacing (10x overall range) of exposure levels were adequate to show results relevant to the outcome of interest (e.g., observation of a dose-response relationship) and the concerns are unlikely to have a substantial impact on results.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable for this study type (metabolite of TCE tested)
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test models were reported with limited descriptive information. Chinese hamster cell lines are routinely used for the outcome of interest. However, these particular pulmonary cell lines (LUC2 and Don.Wg.3H) are not routinely used. At least one of the cell lines does not have a stable karotype (Don.Wg.3H). These limitations are unlikely to have a substantial impact on results.
	Metric 15:	Number per Group	High	× 1	1	The number of replicates per study group (n = 3) was reported and considered consistent with studies of the same or similar type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The outcome assessment methodology was fully described, but cell division aberration is an indirect measure of aneuploidy as chromosome number is not determined by this method.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	At least 100 dividing cells per slide (triplicate; 300 cells) were scored in the cell division assay.
	Metric 19:	Blinding of Assessors	High	× 1	1	Authors reported that slides were independently coded prior to scoring
Domain 6: Confounding / Variable Control						

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Study Citation:	T. Warr, E. Parry, J. Parry (1993). A comparison of two in vitro mammalian cell cytogenetic assays for the detection of mitotic aneuploidy using 10 known or suspected aneugens Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 287(1,1), 29-46
Data Type:	Aneuploidy assay for CH (cell division aberration)
HERO ID:	708375

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among study group parameters (e.g., test substance, cells used) that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported but this deficiency is not likely to significantly impact results
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were described ("control and treated cultures compared using Chi squared analysis and Fisher's exact test [for numbers below 10] at the 95% confidence limit").
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Scoring and/or evaluation criteria were cited to another publication (Parry et al. 1985)
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity was noted with some results suggesting that the authors considered it, but the method of measurement was not reported.
	Metric 25:	Reporting of Data	Low	× 2	6	Data for exposure-related findings were not shown for both cell types (DON:Wg3h cells but LUC2 cells), but results for both cell types were described in the text. These deficiencies impact the results (results shown only for the cell line with an unstable karotype).
Overall Quality Determination‡			High		1.7	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 159: **In vitro evaluation results for Kappas 1989 for fungal mitotic segregation study**

Study Citation:	A. Kappas (1989). On the mechanisms of induced aneuploidy in Aspergillus nidulans and validation of tests for genomic mutations Progress in Clinical and BIological Research, 318 377-384
Data Type:	Mitotic segregation in Aspergillus for chloral hydrate
HERO ID:	714513

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate.
	Metric 2:	Test Substance Source	Medium	× 1	2	Multiple commercial sources for the various test substances were reported, and it was not clear which test substance originated from which source.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (vehicle).
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were appropriate.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design. Metabolic activation with liver S9 was utilized, but not for chloral hydrate.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The identity and genetic features of the strain of Aspergillus were identified. The source of this test model was not identified.
	Metric 15:	Number per Group	High	× 1	1	It was reported that at least 100 colonies were tested at each chemical concentration.
Domain 5: Outcome Assessment						

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Study Citation:	A. Kappas (1989). On the mechanisms of induced aneuploidy in Aspergillus nidulans and validation of tests for genomic mutations Progress in Clinical and BIological Research, 318 377-384
Data Type:	Mitotic segregation in Aspergillus for chloral hydrate
HERO ID:	714513

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methods were cited to other publications, but some details were briefly described and the methods appeared appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	No statistical analysis was conducted, but raw data are available to enable independent analysis.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 24:	Cytotoxicity Data	High	× 1	1	This study was completed in conjunction with a measurement of cytotoxicity (percentage reduction of colony diameter, measured 3 days after inoculation).
	Metric 25:	Reporting of Data	High	× 2	2	All data are reported adequately.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 160: **In vitro evaluation results for Albertini 1990 for mitotic chromosome malsegregation study**

Study Citation:	S. Albertini (1990). Analysis of nine known or suspected spindle poisons for mitotic chromosome malsegregation using Saccharomyces cerevisiae D61.M Mutagenesis, 5(5,5), 453-459
Data Type:	Mitotic chromosome malsegregation for chloral hydrate
HERO ID:	715194

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate with the correct CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (water).
	Metric 5:	Positive Controls	High	× 2	2	Appropriate concurrent positive control test substances were included (bavistan and/or ethylacetate). Positive control groups exhibited positive responses.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration for both incubation protocols was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing was reported and appropriate.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The identity and descriptive information regarding the genetic profile of the yeast strain used here were described. The source was not explicitly stated. More details on the test model developed for this outcome of interest were cited to other references.

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Study Citation:	S. Albertini (1990). Analysis of nine known or suspected spindle poisons for mitotic chromosome malsegregation using Saccharomyces cerevisiae D61.M Mutagenesis, 5(5,5), 453-459
Data Type:	Mitotic chromosome malsegregation for chloral hydrate
HERO ID:	715194

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	Each exposure group included five plates; however, the data from the plates were pooled.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Number of colonies is an objective outcome and blinding assessors is not necessary.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	No statistics were conducted because it appears that n = 1 for all test conditions. Raw data are provided.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of colonies) was reported and consistent with standards and guidelines .
	Metric 24:	Cytotoxicity Data	High	× 1	1	A dose range-finding preliminary trial was conducted. For test substances that were found to be toxic, doses to be tested were selected so that at least one dose was in the non-toxic range and at least three doses were in the toxic range, producing a dose-dependent decrease in survival.
	Metric 25:	Reporting of Data	High	× 2	2	All data are adequately reported.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study.

Table 161: **In vitro evalatuion results for Van Hummelen and Kirsch-Volders 1992 for human lymphocyte micronucleus study**

Study Citation:	P. Van Hummelen, M. Kirsch-Volders (1992). Analysis of eight known or suspected aneugens by the in vitro human lymphocyte micronucleus test Mutagenesis, 7(6,6), 447-455
Data Type:	In vitro micronucleus for chloral hydrate
HERO ID:	720325

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate.
	Metric 2:	Test Substance Source	Medium	× 1	2	The donor source of the test substance was reported. It was not clear whether the chemical was originally obtained from a commercial source of synthesized in-house.
	Metric 3:	Test Substance Purity	Low	× 1	3	The test substance purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control groups were included in the study design.
	Metric 5:	Positive Controls	Medium	× 2	4	Appropriate positive control test substances were included (colchicine and vinblastine), although it was not clear whether these assays were run concurrently.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were reported and appropriate.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design. Although S9 mix was used in this study, it was not tested with chloral hydrate.
Domain 4: Test Model						

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Study Citation:	P. Van Hummelen, M. Kirsch-Volders (1992). Analysis of eight known or suspected aneugens by the in vitro human lymphocyte micronucleus test Mutagenesis, 7(6,6), 447-455
Data Type:	In vitro micronucleus for chloral hydrate
HERO ID:	720325

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Low	× 2	6	The identity of the test model (human lymphocytes) was reported, but many details were lacking, such as demographic information. The only specification was that donors were under 35 years of age.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was completed in duplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The sampling was adequate at 1,000 binucleated lymphocytes per experimental condition.
	Metric 19:	Blinding of Assessors	High	× 1	1	It was reported that the slides were coded prior to analysis.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	The data were analyzed appropriately by Fisher's exact test.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (percentage of cells with micronuclei) is appropriate.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	A toxic dose was reported (800 ug/mL), but it was unclear what methods were utilized to determine this, or whether an assay for cytotoxicity was conducted concurrently with each experimental condition of the micronucleus assay.
	Metric 25:	Reporting of Data	High	× 2	2	All data are reported adequately.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

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Study Citation:	P. Van Hummelen, M. Kirsch-Volders (1992). Analysis of eight known or suspected aneugens by the in vitro human lymphocyte micronucleus test Mutagenesis, 7(6,6), 447-455
Data Type:	In vitro micronucleus for chloral hydrate
HERO ID:	720325

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 162: **Animal toxicity evaluation results Kilgerman et al 1994 for 4-Day inhalation study in rats on cytogenicity**

Study Citation:	A. D. Kligerman, M. F. Bryant, C. L. Doerr, G. L. Erexson, P. A. Evansky, P. Kwanyuen, J. K. Mcgee (1994). Inhalation studies of the genotoxicity of trichloroethylene to rodents Mutation Research, 322(2,2), 87-96
Data Type:	4-Day inhalation cytogenicity studies in rats
HERO ID:	69343

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene (TCE) with the correct CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified. The product number and batch/lot number were not reported; however, the material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported (reagent grade 99+ %)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were tested
	Metric 5:	Positive Controls	Not Rated	NA	NA	The use of positive controls was not applicable for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation methodology was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation was reported. The method and equipment used to generate the test substance as a vapor were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups. For each experiment
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for these endpoints.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The study authors justified the dose concentrations and spacing based on available in vivo cytogenic data .
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route was appropriate for the test substance.
Domain 4: Test Organism						

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Study Citation:	A. D. Kligerman, M. F. Bryant, C. L. Doerr, G. L. Erexson, P. A. Evansky, P. Kwanyuen, J. K. Mcgee (1994). Inhalation studies of the genotoxicity of trichloroethylene to rodents Mutation Research, 322(2,2), 87-96
Data Type:	4-Day inhalation cytogenicity studies in rats
HERO ID:	69343

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and age were reported while health status and starting body weight were not. The test animals were from a reported commercial source. The test species and strain were an appropriate animal model for the evaluation of these endpoints. The uncertainties in reporting are unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most husbandry conditions were reported (temperature, humidity, and chamber conditions). The light-dark cycle was not reported. It was noted that the care and treatment of the animals were approved by the EPA and met all guidelines set by NIH. This limitation is unlikely to have a substantial impact on results.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported, appropriate for the study type and outcome analysis (5/group).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for this study.
	Metric 20:	Negative Control Response	Low	× 1	3	MN in PCE's were elevated in the control group (in comparison to experiments 1,2 and 4 - 1-day exposures); thus, significance could not be properly determined in treated animals. In a separate experiment, an additional control group was included (older male rats 3.5 months of age) to confirm control animal results from this experiment (experiment 3). No significant differences were reported in MN frequency or % PCE between young and old control animals.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food/water intake, and respiratory rate were not reported. These deficiencies are likely to have a substantial impact on results. Trichloroethylene is expected to be a respiratory irritant. The 2-month age range in control animals is also considered a confounding variable.

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Study Citation:	A. D. Kligerman, M. F. Bryant, C. L. Doerr, G. L. Erexson, P. A. Evansky, P. Kwanyuen, J. K. Mcgee (1994). Inhalation studies of the genotoxicity of trichloroethylene to rodents Mutation Research, 322(2,2), 87-96
Data Type:	4-Day inhalation cytogenicity studies in rats
HERO ID:	69343

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 22: Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for the dataset.
	Metric 24: Reporting of Data	High	× 2	2	All data were adequately reported.
Overall Quality Determination‡		High		1.4	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 163: **In vitro evaluation results for Hu et al 2008 for comet assay study**

Study Citation:	C. Hu, L. Jiang, C. Geng, X. Zhang, J. Cao, L. Zhong (2008). Possible involvement of oxidative stress in trichloroethylene-induced genotoxicity in human HepG2 cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 652(1,1), 88-94
Data Type:	Comet assay for TCE
HERO ID:	729534

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene (TCE).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be at least 99.5% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls (DMSO) were included.
	Metric 5:	Positive Controls	High	× 2	2	An appropriate concurrent positive control (hydrogen peroxide), was included. Results were not included in Figure 1 but were described numerically in the text.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were reported and appropriate.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 4: Test Model						

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Study Citation:	C. Hu, L. Jiang, C. Geng, X. Zhang, J. Cao, L. Zhong (2008). Possible involvement of oxidative stress in trichloroethylene-induced genotoxicity in human HepG2 cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 652(1,1), 88-94
Data Type:	Comet assay for TCE
HERO ID:	729534

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The identity and commercial source of the HepG2 cell line was reported. This strain is not routinely used for this endpoint, but rationale was provided for this choice. Other details regarding the cell line, such as doubling time and passage numbers were not included, but this is not likely to have had a substantial impact on results.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was completed in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The sampling was adequate at 150 "randomly selected comets" per experimental condition.
	Metric 19:	Blinding of Assessors	High	× 1	1	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	It was reported that statistical analysis was conducted with one-way ANOVA and Student's t-test. It is not clear which test was used for the Comet assay data. Data could potentially be re-analyzed by estimation of means and standard deviations from Figure 1.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (length of comet tails) is appropriate.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The Comet assay was completed in conjunction with a measurement of cytotoxicity (MTT assay).
	Metric 25:	Reporting of Data	High	× 2	2	All data are reported adequately.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	C. Hu, L. Jiang, C. Geng, X. Zhang, J. Cao, L. Zhong (2008). Possible involvement of oxidative stress in trichloroethylene-induced genotoxicity in human HepG2 cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 652(1,1), 88-94
Data Type:	Comet assay for TCE
HERO ID:	729534

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 164: **In vitro evaluation results for Hu et al 2008 for micronucleus study**

Study Citation:	C. Hu, L. Jiang, C. Geng, X. Zhang, J. Cao, L. Zhong (2008). Possible involvement of oxidative stress in trichloroethylene-induced genotoxicity in human HepG2 cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 652(1,1), 88-94
Data Type:	In vitro micronucleus for TCE
HERO ID:	729534

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene (TCE).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be at least 99.5% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls (DMSO) were included.
	Metric 5:	Positive Controls	High	× 2	2	An appropriate concurrent positive control (cyclophosphamide), was included. Results were not included in Figure 2 but were described numerically in the text.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were reported and appropriate.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 4: Test Model						

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Study Citation:	C. Hu, L. Jiang, C. Geng, X. Zhang, J. Cao, L. Zhong (2008). Possible involvement of oxidative stress in trichloroethylene-induced genotoxicity in human HepG2 cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 652(1,1), 88-94
Data Type:	In vitro micronucleus for TCE
HERO ID:	729534

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The identity and commercial source of the HepG2 cell line was reported. This strain is not routinely used for this endpoint, but rationale was provided for this choice. Other details regarding the cell line, such as doubling time and passage numbers were not included, but this is not likely to have had a substantial impact on results.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was completed in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The sampling was adequate at 1,000 binucleated cells per experimental condition.
	Metric 19:	Blinding of Assessors	High	× 1	1	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	It was reported that statistical analysis was conducted with one-way ANOVA and Student's t-test. It is not clear which test was used for the micronucleus data. Data could potentially be re-analyzed by estimation of means and standard deviations from Figure 2.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (proportion of cells with micronuclei) is appropriate.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The micronucleus assay was completed in conjunction with a measurement of cytotoxicity (MTT assay).
	Metric 25:	Reporting of Data	High	× 2	2	All data are reported adequately.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	C. Hu, L. Jiang, C. Geng, X. Zhang, J. Cao, L. Zhong (2008). Possible involvement of oxidative stress in trichloroethylene-induced genotoxicity in human HepG2 cells Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 652(1,1), 88-94
Data Type:	In vitro micronucleus for TCE
HERO ID:	729534

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 165: **In vitro evaluation results for Bonatti et al 1992 for micronucleus study**

Study Citation:	S. Bonatti, Z. Cavalieri, S. Viaggi, A. Abbondandolo (1992). The analysis of 10 potential spindle poisons for their ability to induce CREST-positive micronuclei in human diploid fibroblasts Mutagenesis, 7(2,2), 111-114
Data Type:	Mammalian CREST/micronucleus assay for chloral hydrate
HERO ID:	729551

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate.
	Metric 2:	Test Substance Source	Not Rated	NA	NA	The commercial source of the test substance was cited to another reference.
	Metric 3:	Test Substance Purity	Not Rated	NA	NA	Details regarding test substances were cited to other references.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (DMSO).
	Metric 5:	Positive Controls	High	× 2	2	Appropriate positive controls were included, though not concurrently with the test substance. A positive response was induced by the positive controls (colchicine and vinblastine).
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate for the study design.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were reported and appropriate.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 4: Test Model						

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Study Citation:	S. Bonatti, Z. Cavalieri, S. Viaggi, A. Abbondandolo (1992). The analysis of 10 potential spindle poisons for their ability to induce CREST-positive micronuclei in human diploid fibroblasts Mutagenesis, 7(2,2), 111-114
Data Type:	Mammalian CREST/micronucleus assay for chloral hydrate
HERO ID:	729551

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	The identity and chromosome number of the two human fibroblast strains (diploid) and V79 Chinese hamster cell line (heteroploid) were reported and appropriate. Some test model details were lacking, such as more information on the human donors of the fibroblasts (age, sex, health status), organ of origin, and passage number. It was noted that the diploid status of the human fibroblasts was confirmed at 4 week intervals throughout the study.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was conducted in duplicate. Certain exposure conditions were conducted in both human fibroblast strains as well as the V79 hamster fibroblasts.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The sampling was adequate at 3,000 intact interphase cells per experimental condition.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initials conditions were not reported for each group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	The data were analyzed appropriately using Chi-square test and Fisher's exact test.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (percentage of cells with micronuclei and ratio of CREST-positive to CREST-negative micronuclei) are consistent with current standards.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The assay was completed in conjunction with a measurement of cytotoxicity (mitotic index).
	Metric 25:	Reporting of Data	High	× 2	2	All data are adequately reported.
Overall Quality Determination‡			High		1.2	

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Study Citation:	S. Bonatti, Z. Cavalieri, S. Viaggi, A. Abbondandolo (1992). The analysis of 10 potential spindle poisons for their ability to induce CREST-positive micronuclei in human diploid fibroblasts Mutagenesis, 7(2,2), 111-114
Data Type:	Mammalian CREST/micronucleus assay for chloral hydrate
HERO ID:	729551

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 166: **In vitro evaluation results for Nesslany and Marzin 1999 for micronucleus study**

Study Citation:	F. Nesslany, D. Marzin (1999). A micromethod for the in vitro micronucleus assay Mutagenesis, 14(4,4), 403-410					
Data Type:	In vitro micronucleus for chloral hydrate					
HERO ID:	729564					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The test substance purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Negative control groups were included, although it is unclear whether these were treated with vehicle or left untreated, and it is unclear whether the negative controls were run concurrently with the chloral hydrate samples.
	Metric 5:	Positive Controls	High	× 2	2	Appropriate concurrent positive control test substances were included without S9 activation (mitomycin C).
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing were reported and appropriate.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design. Although S9 mix was used in this study, it was not tested with chloral hydrate.
Domain 4: Test Model						

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Study Citation:	F. Nesslany, D. Marzin (1999). A micromethod for the in vitro micronucleus assay Mutagenesis, 14(4,4), 403-410
Data Type:	In vitro micronucleus for chloral hydrate
HERO ID:	729564

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The identity, commercial source, and doubling time of the L5178Y TK+/- clone 3.7.2C mouse lymphoma cells were identified. The strain was regularly checked for karyotypic stability and prevalence of polyploid cells.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was completed in duplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The sampling was adequate at 1,000 intact interphase cells per experimental condition.
	Metric 19:	Blinding of Assessors	High	× 1	1	It was reported that the slides were coded prior to analysis.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	The data were analyzed appropriately by Chi-square test. A positive result was defined as a dose-related increase coupled with a statistically significant increase over control in at least one dose. Raw data were provided that would enable an independent statistical analysis.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (percentage of cells with micronuclei) is appropriate.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The micronucleus assay was completed in conjunction with a measurement of cytotoxicity (MTT assay).
	Metric 25:	Reporting of Data	High	× 2	2	All data are reported adequately.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	F. Nesslany, D. Marzin (1999). A micromethod for the in vitro micronucleus assay Mutagenesis, 14(4,4), 403-410
Data Type:	In vitro micronucleus for chloral hydrate
HERO ID:	729564

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 167: **In vitro evaluation results for Vamvakas et al 1993 for c-fos and c-myc expression study**

Study Citation:	S. Vamvakas, D. Bittner, U. Köster (1993). Enhanced expression of the protooncogenes c-myc and c-fos in normal and malignant renal growth Toxicology Letters, 67(1-3,1-3), 161-172					
Data Type:	expression of c-fos and c-myc protooncogenes					
HERO ID:	730039					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by established nomenclature.
	Metric 2:	Test Substance Source	Low	× 1	3	Omitted details on the source of the test substance and/or analytical verification of a synthesized test substance are likely to have a substantial impact on the results.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	A negative control group was indicated, but no details were provided.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 6:	Assay Procedures	Medium	× 1	2	The assay procedures were briefly described but several assay conditions (cell density, temperature, humidity) were not reported.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable for the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of test substance was reported (addition to medium). Storage was not reported but is unlikely to affect the results of this short duration study.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were inferred from the text and appeared to be administered consistent across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity (uM).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Time course studies were performed.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Only a single concentration was used.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	A mammalian cell line was used (renal cells) and a metabolite of TCE was tested
Domain 4: Test Model						

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Study Citation: S. Vamvakas, D. Bittner, U. Köster (1993). Enhanced expression of the protooncogenes c-myc and c-fos in normal and malignant renal growth Toxicology Letters, 67(1-3,1-3), 161-172
Data Type: expression of c-fos and c-myc protooncogenes
HERO ID: 730039

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Low	× 2	6	The test model was reported but no additional details or source were reported.
	Metric 15:	Number per Group	Unacceptable	× 1	4	The number of organisms or tissues per study group and/or replicates per study group were not reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Details regarding the performance of the study were not reported.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial strain/batch/lot number of organisms or models used per group, size, and/or quality of tissues exposed was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analysis was not conducted but is not necessarily required for gene expression measured by densitometric analysis
	Metric 23:	Data Interpretation	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported graphically for the outcomes of interest.
Overall Quality Determination‡			Unacceptable⋆⋆		2.2	
Extracted			No			

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Study Citation:	S. Vamvakas, D. Bittner, U. Köster (1993). Enhanced expression of the protooncogenes c-myc and c-fos in normal and malignant renal growth Toxicology Letters, 67(1-3,1-3), 161-172
Data Type:	expression of c-fos and c-myc protooncogenes
HERO ID:	730039

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 168: **In vitro evaluation results for Zhang et al 2010 for mammalian mutagenicity study**

Study Citation:	S. H. Zhang, D. Y. Miao, A. L. Liu, L. Zhang, W. Wei, H. Xie, W. Q. Lu (2010). Assessment of the cytotoxicity and genotoxicity of haloacetic acids using microplate-based cytotoxicity test and CHO/HGPRT gene mutation assay Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 703(2,2), 174-179
Data Type:	CHO/HGPRT mammalian mutagenicity for MCA, DCA, and TCA
HERO ID:	730076

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified by name as chloroacetic acid (CA, or MCA), dichloroacetic acid (DCA), and trichloroacetic acid (TCA).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substances (Sigma Aldrich) was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substances was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (culture medium).
	Metric 5:	Positive Controls	High	× 2	2	An appropriate concurrent positive control test substance were included with each experimental condition (ethyl methylsulfonate). Positive control groups exhibited expected responses.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation and storage were reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity in uM
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (4 hr) was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and dose spacing was reported and selected based on cytotoxicity assays.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study design.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity and source of the CHO-K1 cell line utilized here was reported.

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Study Citation:	S. H. Zhang, D. Y. Miao, A. L. Liu, L. Zhang, W. Wei, H. Xie, W. Q. Lu (2010). Assessment of the cytotoxicity and genotoxicity of haloacetic acids using microplate-based cytotoxicity test and CHO/HGPRT gene mutation assay Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 703(2,2), 174-179
Data Type:	CHO/HGPRT mammalian mutagenicity for MCA, DCA, and TCA
HERO ID:	730076

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 15: Number per Group	Medium	× 1	2	Initial and confirmatory experiments were performed.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported and is appropriate for the outcome of interest.
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18: Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to this study design
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	High	× 2	2	No differences among treatment group parameters were reported.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported but this deficiency is unlikely to significantly impact the results.
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	High	× 1	1	Homogenous data were analyzed by Dunnett's test and heterogenous data were analyzed using the chi-square test; both were two-tailed. The data were analyzed appropriately.
	Metric 23: Data Interpretation	High	× 2	2	Evaluation criteria (mutant frequency) was reported and consistent with current standards.
	Metric 24: Cytotoxicity Data	High	× 1	1	Both acute and chronic cytotoxicity assays were conducted in addition to the HGPRT mutation assay and cloning efficiency assessed concurrently with the HGPRT assay
	Metric 25: Reporting of Data	High	× 2	2	Results were reported for all outcomes, exposure conditions, and experiments.
Overall Quality Determination‡		High		1.1	
Extracted		Yes			

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Study Citation:	S. H. Zhang, D. Y. Miao, A. L. Liu, L. Zhang, W. Wei, H. Xie, W. Q. Lu (2010). Assessment of the cytotoxicity and genotoxicity of haloacetic acids using microplate-based cytotoxicity test and CHO/HGPRT gene mutation assay Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 703(2,2), 174-179
Data Type:	CHO/HGPRT mammalian mutagenicity for MCA, DCA, and TCA
HERO ID:	730076

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 169: **Animal toxicity evaluation results Kilgerman et al 1994 for 6-hour inhalation study in rats on cytogenicity**

Study Citation:	A. D. Kligerman, M. F. Bryant, C. L. Doerr, G. L. Erexson, P. A. Evansky, P. Kwanyuen, J. K. Mcgee (1994). Inhalation studies of the genotoxicity of trichloroethylene to rodents Mutation Research, 322(2,2), 87-96
Data Type:	Three 6-hour inhalation cytogenicity studies in rats and mice
HERO ID:	69343

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene (TCE) with the correct CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified. The product number and batch/lot number were not reported; however, the material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported (reagent grade 99+ %)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were tested
	Metric 5:	Positive Controls	Not Rated	NA	NA	The use of positive controls was not applicable for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation methodology was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation was reported. The method and equipment used to generate the test substance as a vapor were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups for each experiment.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Analytical concentrations were provided and did not deviate widely.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for these endpoints.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The study authors justified the dose concentrations and spacing based on available in vivo cytogenic data .
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route was appropriate for the test substance.
Domain 4: Test Organism						

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Study Citation:	A. D. Kligerman, M. F. Bryant, C. L. Doerr, G. L. Erexson, P. A. Evansky, P. Kwanyuen, J. K. Mcgee (1994). Inhalation studies of the genotoxicity of trichloroethylene to rodents Mutation Research, 322(2,2), 87-96
Data Type:	Three 6-hour inhalation cytogenicity studies in rats and mice
HERO ID:	69343

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and age were reported while health status and starting body weight were not. The test animals were from a reported commercial source. The test species and strain were an appropriate animal model for the evaluation of these endpoints. The uncertainties in reporting are unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most husbandry conditions were reported (temperature, humidity, and chamber conditions). The light-dark cycle was not reported. It was noted that the care and treatment of the animals were approved by the EPA and met all guidelines set by NIH. This limitation is unlikely to have a substantial impact on results.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported, appropriate for the study type and outcome analysis (5/group).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for this study.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control groups were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food/water intake, and respiratory rate were not reported. These deficiencies are likely to have a substantial impact on results. Trichloroethylene is expected to be a respiratory irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for the dataset.
	Metric 24:	Reporting of Data	High	× 2	2	All data were adequately reported.
Overall Quality Determination[‡]			High		1.4	

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Study Citation:	A. D. Kligerman, M. F. Bryant, C. L. Doerr, G. L. Erexson, P. A. Evansky, P. Kwanyuen, J. K. Mcgee (1994). Inhalation studies of the genotoxicity of trichloroethylene to rodents Mutation Research, 322(2,2), 87-96
Data Type:	Three 6-hour inhalation cytogenicity studies in rats and mice
HERO ID:	69343

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 170: **In vitro evaluation results for Parry et al 1990 for mitotic division aberrations study**

Study Citation:	Parry, J. M., Parry, E. M., Warr, T., Lynch, A., James, S. (1990). The detection of aneugens using yeasts and cultured mammalian cells Progress in Clinical and Biological Research, 340B 247-266
Data Type:	mitotic division aberrations
HERO ID:	733486

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chloral hydrate (TCE metabolite), no CASRN provided.
	Metric 2:	Test Substance Source	Low	× 1	3	Test chemical was provided by the coordinating laboratory. No analytical verification was provided
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity is not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Details of the negative control were not described (untreated vs. vehicle)
	Metric 5:	Positive Controls	High	× 2	2	A positive control was included (colcemid).
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described with acceptable details
	Metric 7:	Standards for Tests	Not Rated	NA	NA	No applicable to the study design
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	No information on test substance preparation and storage was provided.
	Metric 9:	Consistency of Exposure Administration	Low	× 1	3	Exposure administration was described and consistency across groups was inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were clearly reported
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration (24hrs) was reported and appropriate for the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	Three test concentrations were evaluated.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not necessary for the outcome of interest.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Two Chinese hamster test models: immortal DON cells and primary LUC cells were used.
	Metric 15:	Number per Group	High	× 1	1	The study reports scoring three replicates/treatment group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment was adequately described and appropriate for the outcome of interest. Some details were described in another publication.

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Study Citation:	Parry, J. M., Parry, E. M., Warr, T., Lynch, A., James, S. (1990). The detection of aneugens using yeasts and cultured mammalian cells Progress in Clinical and Biological Research, 340B 247-266
Data Type:	mitotic division aberrations
HERO ID:	733486

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistency of outcome assessment is inferred from the text.
	Metric 18:	Sampling Adequacy	High	× 2	2	300 cells were classified/ treatment.
	Metric 19:	Blinding of Assessors	High	× 1	1	Slides were coded.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	The study recognized potential confounding variables and took measures to address these.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Confounding variable in outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	Details of the Statistical analysis applied was not described. Significance at 95% confidence limits were reported.
	Metric 23:	Data Interpretation	High	× 2	2	Positive results were based on statistical significance.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	The study reported that no increase in toxicity was observed, however no details on the assessment of cytotoxicity were provided.
	Metric 25:	Reporting of Data	Medium	× 2	4	Numerical data for only one test model (DON cells) was reported, results for LUC cells were provided as a summary only (+ or -).
Overall Quality Determination‡			~~Unacceptable~~⋆⋆ ⟶ Low§		~~1.6~~	
Extracted			Yes			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Although details of the the test substance preparation and storage were not reported. The remaining metrics were generally scored as medium to high and the positive results may provide important information for this test substance."

Table 171: **In vitro evaluation results for Parry et al 1990 for micronucleus study**

Study Citation:	Parry, J. M., Parry, E. M., Warr, T., Lynch, A., James, S. (1990). The detection of aneugens using yeasts and cultured mammalian cells Progress in Clinical and Biological Research, 340B 247-266
Data Type:	Micronuclei - in vitro
HERO ID:	733486

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chloral hydrate (TCE metabolite), no CASRN provided.
	Metric 2:	Test Substance Source	Low	× 1	3	Test chemical was provided by the coordinating laboratory. No analytical verification was provided
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity is not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Untreated and vehicle (DMSO) controls were included
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were included (MMC)
	Metric 6:	Assay Procedures	Medium	× 1	2	Multiple procedures/protocols were used and described with some detail. Additional details were cited to another publication
	Metric 7:	Standards for Tests	Not Rated	NA	NA	No applicable to the study design
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	No information on test substance preparation and storage was provided.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposure administration was described and consistency across groups was inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were clearly reported
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	Exposure durations were not explicitly reported, but were indicated to be "various periods longer than one cell cycle - 18hrs)"
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (>4) and spacing were appropriate for the outcome of interest.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The study indicated that metabolic activation with liver s9 was used if no positive results were obtained in the absence of activation.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Chinese Hamster primary cultures.
	Metric 15:	Number per Group	High	× 1	1	The study reports scoring three replicates/treatment group
Domain 5: Outcome Assessment						

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Study Citation:	Parry, J. M., Parry, E. M., Warr, T., Lynch, A., James, S. (1990). The detection of aneugens using yeasts and cultured mammalian cells Progress in Clinical and Biological Research, 340B 247-266
Data Type:	Micronuclei - in vitro
HERO ID:	733486

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment was adequately described and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistency of outcome assessment is inferred from the text.
	Metric 18:	Sampling Adequacy	High	× 2	2	>2,000 cells /treatment group
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not reported for this experiment, however other experiments reported in the same study indicated slides were coded. It would be appropriate to assume that coding as also used for the micronuclei studies.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	The study recognized potential confounding variables in the procedures and took measures to address these.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Confounding variable in outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	Details of the Statistical analysis applied was not described. Significance at 95% confidence limits were reported.
	Metric 23:	Data Interpretation	High	× 2	2	Positive results were based on statistical significance.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	The study reported that no increase in toxicity was observed, however no details on the assessment of cytotoxicity were provided.
	Metric 25:	Reporting of Data	High	× 2	2	Data is appropriately reported and available for independent review.
Overall Quality Determination[‡]			~~Unacceptable~~[⋆⋆] ⟶ Low[§]		~~1.5~~	
Extracted			Yes			

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Study Citation:	Parry, J. M., Parry, E. M., Warr, T., Lynch, A., James, S. (1990). The detection of aneugens using yeasts and cultured mammalian cells Progress in Clinical and Biological Research, 340B 247-266
Data Type:	Micronuclei - in vitro
HERO ID:	733486

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i \left(\text{Metric Score}_i \times \text{MWF}_i\right) / \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Although details of the the test substance preparation and storage were not reported. The remaining metrics were generally scored as medium to high and the positive results may provide important information for this test substance."

Table 172: **In vitro evaluation results for Parry et al 1990 for chromosome loss study**

Study Citation:	Parry, J. M., Parry, E. M., Warr, T., Lynch, A., James, S. (1990). The detection of aneugens using yeasts and cultured mammalian cells Progress in Clinical and Biological Research, 340B 247-266
Data Type:	Chromosome loss in Yeast- CH
HERO ID:	733486

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chloral hydrate (TCE metabolite), no CASRN provided.
	Metric 2:	Test Substance Source	Low	× 1	3	Test chemical was provided by the coordinating laboratory. No analytical verification was provided
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity is not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Details of the negative control were not described (untreated vs. vehicle)
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive control was used, however several chemicals were tested and the test substance yielded positive results.
	Metric 6:	Assay Procedures	Low	× 1	3	Assay procedures were described with limited details. Some methods were performed as described in another publication.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	No applicable to the study design
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	No information on test substance preparation and storage was provided.
	Metric 9:	Consistency of Exposure Administration	Low	× 1	3	Details of exposure administration are limited
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	A range of 1,000 to 5,000 ug/mL was reported. Specific doses can be obtained from a graphical figure.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure durations were reported for each experiment
	Metric 12:	Exposure Route and Method	High	× 1	1	A graphical figure indicates 4 doses were used. The concentrations/spacing was appropriate.
	Metric 13:	Metabolic Activation	Low	× 1	3	Liver s9 activation was used in cases where positive results were not obtained with nutrient media however, no descriptive details were provided.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	Strain D6 yeast test model is not routinely used and was described with limited detail. Additional details were cited to another publication.

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Study Citation:	Parry, J. M., Parry, E. M., Warr, T., Lynch, A., James, S. (1990). The detection of aneugens using yeasts and cultured mammalian cells Progress in Clinical and Biological Research, 340B 247-266
Data Type:	Chromosome loss in Yeast- CH
HERO ID:	733486

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	The number of replicates is not reported, however the text mentions repeat experiments.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The outcome assessment methodology was appropriate for the assay performed, but described with very limited details.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Outcome assessment methodology was described with limited details, consistency is unclear.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	The protocol suggested sampling was adequate (at least 200 colonies), however, the results indicates that only 185 colonies were tested.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding of assessors was not reported
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Test model information was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Confounding variable in outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analysis was not described. It is unclear whether statistical analysis was performed, although the text reports that significant increases were observed.
	Metric 23:	Data Interpretation	Low	× 2	6	The text indicates expression of recessive markers at frequencies above 65% are considered positive, however, this is only a 1-fold change above the control (64.5%). It is unclear if this is an acceptable margin.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	The study reported that no increase in toxicity was observed, however no details on the assessment of cytotoxicity were provided.
	Metric 25:	Reporting of Data	Low	× 2	6	Generally, data reporting was acceptable., however it is not clear whether the data provided is from a single test or represents the means from two replicates. No measures of variance were provided.
Overall Quality Determination‡			Unacceptable⋆⋆		2.5	
Extracted			No			

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Study Citation:	Parry, J. M., Parry, E. M., Warr, T., Lynch, A., James, S. (1990). The detection of aneugens using yeasts and cultured mammalian cells Progress in Clinical and Biological Research, 340B 247-266
Data Type:	Chromosome loss in Yeast- CH
HERO ID:	733486

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 173: **In vitro evaluation results for Emmert et al 2006 for Ames test study**

Study Citation:	B. Emmert, J. Bünger, K. Keuch, M. Müller, S. Emmert, E. Hallier, G. A. Westphal (2006). Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S. typhimurium strain YG7108pin3ERb5 Toxicology, 228(1,1), 66-76
Data Type:	Ames assay for TCE
HERO ID:	1006124

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by name as trichloroethylene; the CASRN was provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source (Sigma-Aldrich) was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity (=99.5%) was reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Use of concurrent solvent controls was reported
	Metric 5:	Positive Controls	High	× 2	2	A positive control (N-nitrosodiethylamine) was reported and gave expected results
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were cited to a published study, and partially described
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Slight discrepancies were identified in test substance solution preparation. The methods indicate solutions were prepared in DMSO, however the figure legend indicates the test substance was in ethanol. Test substance storage was not reported.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Details of exposure methods were cited to another publication
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Initial tests with concentrations up to toxic concentrations, 5 mg/plate, or the solubility limit were performed. Specific concentrations in the final test are reported graphically and may be determined from the figures presented, however determining the specific concentrations may be difficult (crowded/overlapping means at lower concentrations)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration for one strain was extended to 72 hrs to account for potential growth delay induced by some compounds.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Based on the figures presented at least 7 concentrations were tested however, significant toxicity was reported at most concentrations so it is unclear if the concentrations tested were appropriate for the evaluating the outcome of interest.

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Study Citation:	B. Emmert, J. Bünger, K. Keuch, M. Müller, S. Emmert, E. Hallier, G. A. Westphal (2006). Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S. typhimurium strain YG7108pin3ERb5 Toxicology, 228(1,1), 66-76
Data Type:	Ames assay for TCE
HERO ID:	1006124

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Medium	× 1	2	Metabolic activation was required for the parent strain and was performed as described in another study, although use of phenobarbital/beta-naphthoflavone induced S9 was reported.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The study used S. typhimurium strain YG7108 (a methyltransferase deficient parent strain) and YG108pin3ERb5, which is a metabolically competent strain. These are non-standard strains for an AMES assay, but were used because they are reported to be more sensitive than normal strains.
	Metric 15:	Number per Group	Medium	× 1	2	The number of strains was lower than the typical number used for this study type however, with the strains used, 3-5 independent experiments were performed.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported (automated culture counting of revertant colonies) and appropriate
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was performed consistently across groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was not conducted, but means and standard deviations are represented in the figures.
	Metric 23:	Data Interpretation	High	× 2	2	Acceptance criteria for a positive test were reported.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Separate cytotoxicity data were not reported, however, cytotoxicity was inferred based on induction of microcolonies with a clear background lawn.

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Study Citation:	B. Emmert, J. Bünger, K. Keuch, M. Müller, S. Emmert, E. Hallier, G. A. Westphal (2006). Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S. typhimurium strain YG7108pin3ERb5 Toxicology, 228(1,1), 66-76
Data Type:	Ames assay for TCE
HERO ID:	1006124

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data		× 2	NA	Results from the parent strain (with and without metabolic activation were not reported. The data presented in the figure lacks clarity (the figure legend indicates it is showing microcolonies, but the graph is labeled as revertants). The text makes a distinction between the two. Based on the information provided, it is unclear if the test substance induced only microcolonies (indicating toxicity), or if revertant colonies were also observed (indicating mutagenicity). The text reports the test substance was negative in the Ames test, but the data does not clearly indicate these results.
Overall Quality Determination‡		Unacceptable⋆⋆		1.6	
Extracted		No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 174: **In vitro evaluation results for Irving and Elfarra 2013 for Ames test study**

Study Citation:	Irving, R.,Elfarra, A. A. (2013). Mutagenicity of the cysteine S-conjugate sulfoxides of trichloroethylene and tetrachloroethylene in the Ames test Toxicology, 306 157-161
Data Type:	Ames assay for TCE metabolites DCVC and DCVCS
HERO ID:	2128042

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name as the TCE metabolites S-(1,2-dichlorovinyl)-l-cysteine DCVC) and S-(1,2-dichlorovinyl)-l-cysteine sulfoxide (DCVCS)
	Metric 2:	Test Substance Source	High	× 1	1	The metabolites were synthesized for the experiment and analytically verified by HPLC
	Metric 3:	Test Substance Purity	High	× 1	1	Purity was reported (>95%)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A negative (buffer) control was used.
	Metric 5:	Positive Controls	Low	× 2	6	A positive control (Sodium azide) was included, however results were not reported.
	Metric 6:	Assay Procedures	High	× 1	1	The assays and procedures relating to exposure were described in detail.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The test chemical was dissolved in buffer and added to the solution. Information on test chemical storage was not reported. For a short-term study this is not expected to significantly influence the results.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Consistent administration across test groups is inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	A concentration range was reported, and specific concentrations can be determined from the dose-response curves provided.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (20 min pre-incubation followed by 48 hrs on a plate) and appropriate for the outcome of interest
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (5 to 13 depending on the metabolite tested) and spacing was reported and appropriate for the outcomes of interest.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not included (TCE metabolites tested directly)
Domain 4: Test Model						

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Study Citation:	Irving, R.,Elfarra, A. A. (2013). Mutagenicity of the cysteine S-conjugate sulfoxides of trichloroethylene and tetrachloroethylene in the Ames test Toxicology, 306 157-161
Data Type:	Ames assay for TCE metabolites DCVC and DCVCS
HERO ID:	2128042

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The test model (S. typhimurium strain TA100) is appropriate and routinely used for the outcome of interest. The commercial source (Bioreliance) was reported
	Metric 15:	Number per Group	Medium	× 1	2	The number of strains tested (1) is lower than the typical number used in studies of a similar type (5). The number of replicates (n=3) for the single strain was appropriate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology (revertant colony count) was described and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistency in outcome assessment between exposure groups and controls was inferred from the text.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study design
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study design
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Data were presented as means ± SEM of 3 replicates. Statistical analysis was performed using the Wilcoxon rank sum test.
	Metric 23:	Data Interpretation	Medium	× 2	4	Statistical significance was used to indicate a positive result. The criteria for the strength of mutagenicity were not reported. The study indicates that "points where toxicity were observed were not included" [in determination of mutagenic activity]. It is not clear how this impacts the results
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Specific assays for cytotoxicity were not included in the study design; however, the text indicated that toxicity was assessed based on microcolony formation or decreasing total number of revertants with increasing concentrations.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported graphically (mean and SE for 3 replicates); positive control data were not reported.

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Study Citation:	Irving, R.,Elfarra, A. A. (2013). Mutagenicity of the cysteine S-conjugate sulfoxides of trichloroethylene and tetrachloroethylene in the Ames test Toxicology, 306 157-161
Data Type:	Ames assay for TCE metabolites DCVC and DCVCS
HERO ID:	2128042

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		High		1.4	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 175: **In vitro evaluation results for Palbykin et al 2011 for DNA methylation study in rat myoblast cells**

Study Citation:	Palbykin, B., Borg, J., Caldwell, P.T., Rowles, J., Papoutsis, A.J., Romagnolo, D.F., Selmin, O.I. (2011). Trichloroethylene induces methylation of the Serca2 promoter in H9c2 cells and embryonic heart Cardiovascular Toxicology, 11(3), 204-214
Data Type:	DNA methylation in rat myoblast cells exposed to TCE
HERO ID:	2128264

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source (Sigma Aldrich) was reported
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were used and kept in a separate incubator to prevent vapor transfer.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were well described with the exception of temperature and humidity. Assay appeared to be appropriate to the outcome of interest.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric was not applicable.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage of test substance were described and methods for preventing loss (e.g., flushing air space in TCE storage containers with nitrogen gas to reduce chemical breakdown) were described.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Administration of exposures were consistent across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported in ppb.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Duration of exposure for each experiment (0.5 to 2 hr) was described.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Two exposure concentrations (1 ppb and 10 ppm) were used. The high concentration was selected based on previous publications.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable to this study type
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (rat cardiomyocyte cell line H9c2) was described and its source reported.
	Metric 15:	Number per Group	Low	× 1	3	Number of cells and/or replicates per exposure group was not reported.
Domain 5: Outcome Assessment						

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Study Citation:	Palbykin, B., Borg, J., Caldwell, P.T., Rowles, J., Papoutsis, A.J., Romagnolo, D.F., Selmin, O.I. (2011). Trichloroethylene induces methylation of the Serca2 promoter in H9c2 cells and embryonic heart Cardiovascular Toxicology, 11(3), 204-214
Data Type:	DNA methylation in rat myoblast cells exposed to TCE
HERO ID:	2128264

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was described and appropriate to the outcome.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Outcomes assessments were administered consistently except that the control was assessed after 2 hr and the treated groups were assessed after 1 and 2 hr.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric was not applicable to study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported but are unlikely to significantly impact the results.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Data manipulation and calculations were described and appropriate. Statistical analysis is not necessarily required for this outcome.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	This metric was not applicable.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric was not applicable.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported graphically for all outcomes.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 176: **In vitro evaluation results for Varshney et al 2013 for micronucleus study**

Study Citation:	Varshney, M.,Chandra, A.,Chauhan, L. K.,Goel, S. K. (2013). Micronucleus induction by oxidative metabolites of trichloroethylene in cultured human peripheral blood lymphocytes: a comparative genotoxicity study Environmental Science and Pollution Research, 20(12), 8709-8716
Data Type:	Micronucleus Assay
HERO ID:	2129572

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chloral hydrate, a TCE metabolite. CASRN provided.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source reported
	Metric 3:	Test Substance Purity	High	× 1	1	Purity >98%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A vehicle (DMSO) control was included.
	Metric 5:	Positive Controls	High	× 2	2	A positive (EMS) control was included.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures we adequately reported and appropriate for the outcome of interest.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was prepared in DMSO. Storage conditions were not described.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure methods were reported and consistency of exposure is implied from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were clearly reported
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	72hr exposure period was appropriate for the assay type
	Metric 12:	Exposure Route and Method	High	× 1	1	3 test concentrations were tested; justification for doses used was not provided. Cytotoxicity was observed at the mid and high dose.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation is not necessary for the cell type.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Primary human lymphocytes (number of donors not reported). - Isolation and some culture methods were described. Some details were described in another study.
	Metric 15:	Number per Group	Medium	× 1	2	Experiments were run in triplicates
Domain 5: Outcome Assessment						

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Study Citation:	Varshney, M.,Chandra, A.,Chauhan, L. K.,Goel, S. K. (2013). Micronucleus induction by oxidative metabolites of trichloroethylene in cultured human peripheral blood lymphocytes: a comparative genotoxicity study Environmental Science and Pollution Research, 20(12), 8709-8716
Data Type:	Micronucleus Assay
HERO ID:	2129572

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Assessment methodology was adequately reported. Cytchalasin B (cytoB) was not used as an actin polymerization inhibitor and relative population doubling or increase in cell count was not determined; however, cytoxicity, cell cycle distribution and apoptosis for the test groups were evaluated.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was adequately reported. Consistent assessed across test groups is inferred from the text.
	Metric 18:	Sampling Adequacy	High	× 2	2	Micronucleus assays were run in triplicate. The number of cells used was appropriate for the assay type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable. Outcomes were not empirically assessed.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Confounding variables were not reported. The study reported that all of the donors were healthy
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Confounding variables in outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Appropriate statistical analysis was included in the study report
	Metric 23:	Data Interpretation	High	× 2	2	Increased frequencies were determined by statistical significance.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity was evaluated by a commonly used assay (MTT)
	Metric 25:	Reporting of Data	High	× 2	2	Data was clearly and adequately presented for all test groups
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

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Study Citation:	Varshney, M.,Chandra, A.,Chauhan, L. K.,Goel, S. K. (2013). Micronucleus induction by oxidative metabolites of trichloroethylene in cultured human peripheral blood lymphocytes: a comparative genotoxicity study Environmental Science and Pollution Research, 20(12), 8709-8716
Data Type:	Micronucleus Assay
HERO ID:	2129572

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 177: **In vitro evaluation results for Deferme et al 2015 for DNA strand break study**

Study Citation:	Deferme, L.,Wolters, J.,Claessen, S.,Briedé, J.,Kleinjans, J. (2015). Oxidative Stress Mechanisms Do Not Discriminate between Genotoxic and Nongenotoxic Liver Carcinogens Chemical Research in Toxicology, 28(8), 1636-1646
Data Type:	dsDNA breaks and 8-OHdG
HERO ID:	3489972

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name as tetrachloroethylene (TCE).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source (Sigma-Aldrich) was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent solvent (EtOH) controls were reported, but data was not shown.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls (menadione, etoposide) were used when appropriate
	Metric 6:	Assay Procedures	Medium	× 1	2	Assays (gamma H2AX and 8-OHdG) were preformed as previously described or according to the manufacturer protocols. Brief details were provided.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study design
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Limited details of test substance preparation (stock solution diluted into media to desired concentration at the time of the assay) were provided. Test substance storage was not provided, but this is appropriate given the study design (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Time matched controls were reported to be treated in an identical manner as the treatment group
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The concentration used (2mM) was clearly stated
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure durations (24, 48, and 72hr) were clearly reported and appropriate for the outcomes of interest.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The single exposure group was appropriate for the outcome of interest, however, the chosen concentration (reported to be the IC20 concentration based on previous MTT assays after 72hr exposure) was hypothesized to be the optimal dose for seeing gene expression changes which were evaluated in the same study. Since the DNA damage assay results were negative, it is unclear whether this concentration was truly appropriate for these specific outcomes.

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Study Citation: Deferme, L.,Wolters, J.,Claessen, S.,Briedé, J.,Kleinjans, J. (2015). Oxidative Stress Mechanisms Do Not Discriminate between Genotoxic and Nongenotoxic Liver Carcinogens Chemical Research in Toxicology, 28(8), 1636-1646
Data Type: dsDNA breaks and 8-OHdG
HERO ID: 3489972

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not included, but is not necessarily relevant to the outcome of interest.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (Hep2 cells) was adequately described including passage number, commercial source, and detailed culture conditions/confluency prior to the test.
	Metric 15:	Number per Group	Medium	× 1	2	Three replicates were reported for each exposure duration. It was not specified if these were technical or biological replicates.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment was adequately described and appropriate for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were consistently assessed across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	An appropriate number of cells (10,000/sample) were analyzed by flow cytometry.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences between study group parameters. The same lot of cells were used for control and treatment groups.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Confounding variables in outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Appropriate statistical analysis (paired student's T-test) was used to determine differences between control and treatment groups.
	Metric 23:	Data Interpretation	High	× 2	2	Data interpretation was briefly described ("Cells with significant levels of g-H2Ax and 8-OHdG positive signals were presented as a percentage of total cells."); however, more details methods on gating procedures for analyzing flow cytometry results were not presented and may be presented in the cited references. However, the data interpretation appeared appropriate.

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Study Citation:	Deferme, L.,Wolters, J.,Claessen, S.,Briedé, J.,Kleinjans, J. (2015). Oxidative Stress Mechanisms Do Not Discriminate between Genotoxic and Nongenotoxic Liver Carcinogens Chemical Research in Toxicology, 28(8), 1636-1646
Data Type:	dsDNA breaks and 8-OHdG
HERO ID:	3489972

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	The concentration tested was previously determined to be the IC20. – Additional (concurrent) cytotoxicity assays were not performed/reported.
	Metric 25:	Reporting of Data	High	× 2	2	Results for all samples/outcomes were adequately reported. Data was presented in figures (bar graphs) as means with SEM.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 178: **In vitro evaluation results of Shell Oil Co 1980 for a mutagenicity study on unscheduled DNA synthesis in human cells outcomes**

Study Citation:	Shell Oil Company (1980). Initial submission: Teratogenic-mutagenic risk of workplace contaminants: Trichloroethylene, perchloroethylene, & carbon disulfide (final report) with attachments and cover letter
Data Type:	Mutagenicity study for TCE and Perc (UDS, TCE)
HERO ID:	4215763

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source (North Strong) was identified.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity reported to be 99.9% pure by IR spectroscopy
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative vehicle (DMSO) controls were included.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls (N-methyl-N-nitro-N-nitroguanidie in absence S9 and benzo[a]pyrene in presence S9) were included.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were partially reported; cell culture methods were cited to a published paper (Stich and Laishes)
	Metric 7:	Standards for Tests	Not Rated	NA	NA	QC criteria not applicable.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Limited details on preparation (only the solvent used) were reported. Storage details were not provided.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity (ul/ml; Table 37)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration (1.5 hr) was reported and was adequate for the positive controls.
	Metric 12:	Exposure Route and Method	High	× 1	1	4 exposure groups plus controls (0.1, 0.5, 1.0, and 5.0 ul/ml) were used. Doses were not justified but were sufficient to induce a positive result
	Metric 13:	Metabolic Activation	Low	× 1	3	Activation system (S9, not further specified) was reported but details regarding the source and volume used were not reported.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model (Human diploid WI-38 cells from embryonic lung tissue at passage 24) and commercial source (Flow Laboratories) were described

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Study Citation:	Shell Oil Company (1980). Initial submission: Teratogenic-mutagenic risk of workplace contaminants: Trichloroethylene, perchloroethylene, & carbon disulfide (final report) with attachments and cover letter
Data Type:	Mutagenicity study for TCE and Perc (UDS, TCE)
HERO ID:	4215763

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	Medium	× 1	2	The number of cells/cell density was not reported; the number of replicates (duplicate) was reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology (liquid scintillation counting and expression as disintegrations per minute per mass DNA) was described in detail.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to assay type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment; all experiments performed on single batch of cells.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences among the study replicates or groups in test model unrelated to exposure and the test substance did not interfere with the assay
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analysis not necessarily required for this assay
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were reported (values of 150% or more relative to control were considered positive) but were based on laboratory experience rather than more broadly established standards.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity assessment not required.
	Metric 25:	Reporting of Data	Medium	× 2	4	Results appear to be reported as means (of duplicate plates) only (no measure of variability) in Table 37 and Figure 3
Overall Quality Determination[‡]			High		1.3	
Extracted			Yes			

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Study Citation:	Shell Oil Company (1980). Initial submission: Teratogenic-mutagenic risk of workplace contaminants: Trichloroethylene, perchloroethylene, & carbon disulfide (final report) with attachments and cover letter
Data Type:	Mutagenicity study for TCE and Perc (UDS, TCE)
HERO ID:	4215763

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 179: **In vitro evaluation results of Shell Oil Co 1980 for mutagenicity study-drosophila on sex-linked recessive lethal in drosophila outcomes**

Study Citation:	Shell Oil Company (1980). Initial submission: Teratogenic-mutagenic risk of workplace contaminants: Trichloroethylene, perchloroethylene, & carbon disulfide (final report) with attachments and cover letter
Data Type:	Mutagenicity study for TCE and Perc (TCE, Drosophila)
HERO ID:	4215763

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source (North Strong) was identified.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity reported to be 99.9% pure by IR spectroscopy
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls were exposed to filtered air
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were fed sucrose containing ethylmethane sulfonate
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were reported fully
	Metric 7:	Standards for Tests	Not Rated	NA	NA	QC criteria not applicable.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Atmosphere generation was described in detail. Storage of test material was not reported but is not expected to significantly impact study due to short duration (7 hr one time). TCE concentrations in Drosophila exposure chambers were not measured analytically.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups. Controls exposed to filtered air.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported in ppm in Tables 38 and 39 (100 and 500 ppm)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was 7 hr
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups and spacing (2 plus controls, 5x range [100 and 500 ppm]) were reported but not justified.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Activation not relevant to assay
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	Test model (Drosophila) and mating scheme were described. Source of the test animals was not reported. Authors note that the strain employed was not the repair deficient mutant requested by the sponsor (NIOSH).

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Study Citation:	Shell Oil Company (1980). Initial submission: Teratogenic-mutagenic risk of workplace contaminants: Trichloroethylene, perchloroethylene, & carbon disulfide (final report) with attachments and cover letter
Data Type:	Mutagenicity study for TCE and Perc (TCE, Drosophila)
HERO ID:	4215763

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of flies per group was reported (300 day old males, with 200 males used in mating scheme) and appeared appropriate
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported in full.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable
	Metric 19:	Blinding of Assessors	High	× 1	1	Authors reported double blind scoring of negative control and test groups.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters (\that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were reported (Kastenbaum-Bowman test)
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Criteria not required.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity evaluation not applicable
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all treatment groups and outcomes (incidence lethality and loss of X or Y chromosome)
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 180: **Animal toxicity evaluation results of Beliles et al 1980 for a 3-wk gestational inhalation study on genotoxicity in vivo (mechanistic) outcomes**

Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	in vivo genotoxicity
HERO ID:	58331

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name and synonym
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer and lot number given.
	Metric 3:	Test Substance Purity	Medium	× 1	2	91% pure, impurities were not characterized
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Filtered air controls; "To avoid exposure of control animals to test materials, all control chambers were in a different chamber room than the exposure chambers. No test materials were taken into the control rooms."
	Metric 5:	Positive Controls	High	× 1	1	Positive controls (reference mutagens) were used for all studies. "However, the contractor did not attempt to verify the purity of these commercially available samples."
	Metric 6:	Randomized Allocation	High	× 1	1	"The animals were randomly assigned to experimental groups."
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Method and equipment used to generate the test substance as a vapor were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and analytical concentrations were provided. Range of measure concentration did not deviate more than 10% target concentration.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration were reported and appropriate for this study.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 exposure concentrations (100 and 500ppm)
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic chamber , whole body, assumed that chemical does not condense.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain and source were reported; starting age and body weight not given.

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Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	in vivo genotoxicity
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	well reported
	Metric 15:	Number per Group	High	× 1	1	6-10/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Dominant lethal assay, spermhead abnormality, chromosomal aberration in rat bone marrow, rat dominant lethal test conducted.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported, but most outcomes were not subjective.
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	None related to genotoxicity
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	None related to genotoxicity
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistics were well described and appropriate
	Metric 24:	Reporting of Data	High	× 2	2	All outcomes were reported.
Overall Quality Determination‡			High		1.2	
Extracted			No			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 181: **Animal toxicity evaluation results of NTP 1990 for a 13-wk oral study in rats and mice on mortality, nutrition and metabolic/adult exposure body weight outcomes**

Study Citation:	J. S. Yoon, J. M. Mason, R. Valencia, R. C. Woodruff, S. Zimmering (1985). Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the National Toxicology Program Environmental Mutagenesis, 7(3,3), 349-367
Data Type:	Chloral Hydrate sex linked recessive lethal in drosophila
HERO ID:	194373

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Table 1 number 10 Chloral hydrate, CASRN: 302-17-0 and structure included
	Metric 2:	Test Substance Source	High	× 1	1	Test substance source is Chemical Dynamics Corp #I01215 (in table 1). Lot number was not reported; however, the test substance is unlikely to vary in composition
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity reported in table 1: Labeled purity- blank, analyzed purity- 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative concurrent controls were used. It was not reported if the negative controls were vehicle or untreated
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	This metric is not applicable to Drosophila.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was prepared using water as the solvent. Storage was not described but omission of these details is unlikely to have a substantial impact on results (3 day diet and injection).
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	Protocols were from previously cited literature and were not reported in text.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Feeding dose reported in table 2 as 0, 5500 ppm; injection doses are reported as 0, 10,000 ppm.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Feeding study duration was 3 days (assume continuous) and injection was administered (if no mutation with diet)
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Concentration was selected based on solubility, palatability, and toxicity (not further described). Single dose group for each route.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Route is reported as oral dietary study and if no mutation are induced, the chemical is injected. It was not reported whether diet was prepared daily to account for volatility

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Study Citation:	J. S. Yoon, J. M. Mason, R. Valencia, R. C. Woodruff, S. Zimmering (1985). Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the National Toxicology Program Environmental Mutagenesis, 7(3,3), 349-367
Data Type:	Chloral Hydrate sex linked recessive lethal in drosophila
HERO ID:	194373

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Drosophila stocks and mating schemes were not reported in text, but cited in (Woodruff et al, 1984; Zimmering et al, 1984; Valencia et al, 1985). Canton-S males were mated in 3 consecutive harems with Basc females over 7 days
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.in text.
	Metric 15:	Number per Group	High	× 1	1	At least 20 F2 Basc males (or Basc/+ females) were examined. Statistical analysis (power) was not reported but number is consistent with the study type
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Testing protocols and experimental methods were cited in (Woodruff et al, 1984; Zimmering et al, 1984; Valencia et al, 1985).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent in protocol and time across all study groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Details regarding sampling adequacy are not applicable for this study type
	Metric 19:	Blinding of Assessors	High	× 1	1	A blind test for induction of SLRLs
	Metric 20:	Negative Control Response	High	× 1	1	The biological response in the negative control group was adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Palatability was reported to be part of the dose selection process but is not further described.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was not conducted, however, sufficient data were provided to allow for other statistical tests.
	Metric 24:	Reporting of Data	High	× 2	2	Quantitative data are reported in table 2 by dose group and summary data are reported in table 4
Overall Quality Determination‡			Medium		1.7	
Extracted			Yes			

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Study Citation:	J. S. Yoon, J. M. Mason, R. Valencia, R. C. Woodruff, S. Zimmering (1985). Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the National Toxicology Program Environmental Mutagenesis, 7(3,3), 349-367
Data Type:	Chloral Hydrate sex linked recessive lethal in drosophila
HERO ID:	194373

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 182: **Animal toxicity evaluation results for Millman et al 1988 for acute oral study in rats on liver outcomes**

Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	TCE GGT+ foci initiation and promotion protocols
HERO ID:	200479

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by chemical name.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer was specified.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was reported as a range for multiple compounds (97-99% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Vehicle controls were used (corn oil).
	Metric 5:	Positive Controls	High	× 1	1	Diethylnitrosamine initiation followed by phenobarbital promotion was utilized as a positive control and was appropriate for the outcome of interest. Positive controls yielded positive responses.
	Metric 6:	Randomized Allocation	High	× 1	1	Randomization was indicated.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation in corn oil was indicated, but storage was not described.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Gavage volume was indicated and appropriate.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	MTD doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for the initiation/promotion study types.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	A single dose was used (specified as the MTD).
	Metric 12:	Exposure Route and Method	High	× 1	1	Oral gavage in corn oil is appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source of the test animal, age and health status were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	High	× 1	1	9-10 rats/group
Domain 5: Outcome Assessment						

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Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	TCE GGT+ foci initiation and promotion protocols
HERO ID:	200479

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Due to incomplete reporting, it was unclear whether methods were sensitive for the outcome of interest. Staining procedures were not described (cited to another publication).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Animals were sacrificed at a consistent timepoint.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Livers were examined for all exposed animals. It appears that only one slide per liver was assessed. The standard deviation values in Tables 3 and 4 represent variation across square centimeters of the tissue.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not required for initial histopathology evaluation.
	Metric 20:	Negative Control Response	High	× 1	1	Negative controls responded appropriately.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food/water consumption were not reported for each study group.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was not described. However, sufficient summary data is provided, enabling independent statistical analysis.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for each exposure group.
Overall Quality Determination‡			High		1.7	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 183: **Animal toxicity evaluation results of Mirsalis et al 1989 for unscheduled DNA synthesis in vivo**

Study Citation:	J. C. Mirsalis, C. K. Tyson, K. L. Steinmetz, E. K. Loh, C. M. Hamilton, J. P. Bakke, J. W. Spalding (1989). Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: Testing of 24 compounds Environmental and Molecular Mutagenesis, 14(3,3), 155-164
Data Type:	UDS in vivo for TCE
HERO ID:	200781

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Two concurrent solvent control groups were included (water and corn orl gavage) for rats. Only a corn oil control group was included for mice. TCE was administered by corn oil gavage.
	Metric 5:	Positive Controls	High	× 1	1	Dimethylnitrosamine and 2-acetylaminofluorene were included as positive controls. Positive responses were observed from positive controls.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation of the test substance was briefly reported. Storage of the test substance was not reported, but this is appropriate given the acute timeframe of the study.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was reported to be consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration were reported and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose spacing was appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, commercial source, and starting body weight range of the test animals were reported. Age of the test animals was not reported.

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Study Citation:	J. C. Mirsalis, C. K. Tyson, K. L. Steinmetz, E. K. Loh, C. M. Hamilton, J. P. Bakke, J. W. Spalding (1989). Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: Testing of 24 compounds Environmental and Molecular Mutagenesis, 14(3,3), 155-164
Data Type:	UDS in vivo for TCE
HERO ID:	200781

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported and appropriate.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and appropriate for these endpoints (n = 3 for all rat and mouse TCE-treated groups; n = 2 for rat corn oil controls at 2 hr; n = 52 for rat corn oil controls at 12 hours; n = 31 for rat water controls at 2 hours; n = 4-13 for male and female mouse corn oil controls at 2 and 12 hr).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for this endpoint.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Fifty cells per slide and 3 slides per animal were assessed.
	Metric 19:	Blinding of Assessors	High	× 1	1	The slides were coded prior to analysis.
	Metric 20:	Negative Control Response	High	× 1	1	Negative responses were observed in negative controls.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Starting body weight ranges were included. Food and water consumption and respiratory rates were not reported, but this is appropriate given the study design.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No deaths or health effects unrelated to the test substance administration were observed.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	No statistical analysis was performed on the data. A positive result was defined as an average net nuclear grain count exceeding 0, which was reported to be in line with the lab's historical controls (negative controls never exceeding an average net nuclear grain count of 0). These criteria are appropriate for the outcome of interest. Statistical analysis could be conducted based on the summary data (means, SEM, and n) provided in Tables I and II.
	Metric 24:	Reporting of Data	High	× 2	2	The data were reported adequately.

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Study Citation:	J. C. Mirsalis, C. K. Tyson, K. L. Steinmetz, E. K. Loh, C. M. Hamilton, J. P. Bakke, J. W. Spalding (1989). Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: Testing of 24 compounds Environmental and Molecular Mutagenesis, 14(3,3), 155-164
Data Type:	UDS in vivo for TCE
HERO ID:	200781

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		High		1.2	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 184: **Animal toxicity evaluation results for Keller and Heck 1988 for DNA binding in rats**

Study Citation:	D. A. Keller, H. Heck (1988). Mechanistic studies on chloral toxicity: Relationship to trichloroethylene carcinogenesis Toxicology Letters, 42(2,2), 183-191
Data Type:	In vivo DNA binding for chloral/chloral hydrate
HERO ID:	628835

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Low	× 2	6	The test substance was identified as chloral (trichloroacetaldehyde) as well as chloral hydrate. These two terms were used interchangeably throughout the article. Chloral is readily converted to chloral hydrate in the presence of water.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of chloral hydrate was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	This metric is not applicable measurement of radiolabeled test compound is the outcome).
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 6:	Randomized Allocation	Low	× 1	3	No random allocation of animals was reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported. Storage of the test substance was not reported (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was reported to be consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration were reported and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Not Rated	NA	NA	This metric is not applicable to the study design, as only one dose of chloral hydrate was utilized.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, and commercial source of the test animals were reported. The age and starting body weight range of the test animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were briefly reported and appropriate.

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Study Citation: D. A. Keller, H. Heck (1988). Mechanistic studies on chloral toxicity: Relationship to trichloroethylene carcinogenesis Toxicology Letters, 42(2,2), 183-191
Data Type: In vivo DNA binding for chloral/chloral hydrate
HERO ID: 628835

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and appropriate for these endpoints (n = 5).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for this endpoint.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable for the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 20:	Negative Control Response	Not Rated	NA	NA	This metric is not applicable to the study design, as no negative controls were reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Starting body weight range was not included.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	It is unclear what statistical analyses were conducted on the data.
	Metric 24:	Reporting of Data	Unacceptable	× 2	8	Data reporting was inadequate. Data from the in vivo portion of the study were only reported in the text (no figures or tables) and no numbers were used to quantify DNA binding endpoints (e.g. "[T]here was a small amount of radioactivity associated with the DNA of all mice treated with [14C]chloral, both TCE-treated and control. Radioactivity in the IF-DNA was barely detectable.") This renders the study unusable.
Overall Quality Determination‡			Unacceptable⋆⋆		2.0	
Extracted			No			

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Study Citation:	D. A. Keller, H. Heck (1988). Mechanistic studies on chloral toxicity: Relationship to trichloroethylene carcinogenesis Toxicology Letters, 42(2,2), 183-191
Data Type:	In vivo DNA binding for chloral/chloral hydrate
HERO ID:	628835

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 185: **Animal toxicity evaluation results of Beliles et al 1980 for a 3-wk gestational inhalation study on genotoxicity in vivo (mechanistic) outcomes**

Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	in vivo genotoxicity /SLRL Drosophila for TCE
HERO ID:	58331

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name and synonym
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer and lot number given.
	Metric 3:	Test Substance Purity	High	× 1	1	99.9% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Filtered air controls reported
	Metric 5:	Positive Controls	High	× 1	1	Positive control (ethylmethane sulfonate) was used. "However, the contractor did not attempt to verify the purity of these commercially available samples."
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	Not applicable for Drosophila assay
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Method and equipment used to generate the test substance as a vapor were reported and appropriate. "Trichloroethylene exposure atmospheres were generated by bubbling dry, oil free air through a column of technical grade Trichloroethylene in a fritted glass, gas wash bottle. The concentrated vapor was introduced into the dilution air stream. "
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported. "Drosophila for the Sex-Linked Recessive Lethal Test were exposed in small cone-shaped cages constructed of fine mesh stainless steel screening material. The base of the cone was about 2.5 inches in diameter and tapered to a 1.25-inch mouth, 4 inches above the base. The mouth of the cone was closed with a cotton plug"

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Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	in vivo genotoxicity /SLRL Drosophila for TCE
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Because sampling in a cage with flies was not considered feasible, only nominal concentrations were reported. Test sampling in an empty fly cage placed inside an animal exposure chamber was performed for Perc but not for TCE. This showed concentrations in the cage to be 84.53% of the concentrations in the chamber; difference was attributed to turbulence in cage caused by sampling.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration were reported and appropriate for this study (7 hr)
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 exposure concentrations (100 and 500ppm)
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic chamber; assumed that chemical does not condense at tested concentrations.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Species and strain /mating scheme described in detail; source not reported. Authors note that the TCE assay did not use repair deficient mutants requested by NIOSH.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Well reported except for humidity:"The Drosophila stocks were maintained at 25°C in glass vials. The culture medium used was Carolina Biological Instant Drosophila Medium (Formula 4-24. without dyes). Flies were immobilized with filtered CO2 for handling"
	Metric 15:	Number per Group	High	× 1	1	300 males/group exposed
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	SLRL and X and Y chromosome loss assays. Assessment methods partially reported; mating scheme cited to another publication.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to this assay
	Metric 19:	Blinding of Assessors	High	× 1	1	"Double blind scoring of the negative control and the test material was performed."
	Metric 20:	Negative Control Response	High	× 1	1	Negative control response was reported and appeared to be appropriate
Domain 6: Confounding / Variable Control						

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Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	in vivo genotoxicity /SLRL Drosophila for TCE
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among the study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistics were described and appropriate: "The data were statistically analyzed by the Kastenbaum-Bowman test"
	Metric 24:	Reporting of Data	High	× 2	2	All outcomes were reported for all groups.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 186: **Animal toxicity evaluation results for Chang et al 1992 for DNA damage study in mouse gastrointestinal cells**

Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Stomach/duodenum DNA damage in mice given single dose MCA, DCA, or TCA
HERO ID:	628837

Domain	Metric		Rating†	MWF*	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified by name as trichloroacetic acid (TCA), dichloroacetic acid (DCA), and monochloroacetic acid (MCA).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substances was reported (Sigma)
	Metric 3:	Test Substance Purity	Low	× 1	3	The purities of the test substances were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle control groups were included (water).
	Metric 5:	Positive Controls	High	× 1	1	Concurrent positive controls (methyl methanesulfonate) were included in the study design.
	Metric 6:	Randomized Allocation	Low	× 1	3	The method of allocation of animals to treatment groups was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported. Storage of the test substance was not reported but is unlikely to significantly impact results (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was reported to be consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (10 mmol/kg)
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration (single administration) were reported and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	A single exposure group for each test substance (10 mmol/kg of TCA, DCA, or MCA) was included.Doses were selected based on lethality, carcinogenicity, or DNA damage in prior studies.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure (gavage) were appropriate for the test substance.
Domain 4: Test Organism						

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Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Stomach/duodenum DNA damage in mice given single dose MCA, DCA, or TCA
HERO ID:	628837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, commercial source, and starting body weight range of the test animals were reported. The age and initial health status of the test animals were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported, appropriate, and consistent across groups.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per treatment group was lower than recommended for these endpoints (n = 4 for DCA and TCA; 2/group for MCA).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (alkaline unwinding) was appropriate for this endpoint. It was described thoroughly.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcome of interest (3 technical replicates per animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest (determined flourometrically)
	Metric 20:	Negative Control Response	High	× 1	1	Responses in negative controls were reported and appeared to be appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Starting body weight ranges were included. Food and water consumption and respiratory rates were not reported, but this is not expected to significantly impact the results given the short study duration and gavage administration.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The data were analyzed appropriately by Dunnett's multiple comparison test.
	Metric 24:	Reporting of Data	High	× 2	2	Results were reported for all treatment groups, including mean, SE, and n.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Stomach/duodenum DNA damage in mice given single dose MCA, DCA, or TCA
HERO ID:	628837

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 187: **Animal toxicity evaluation results for Chang et al 1992 for DNA damage study in mouse and rat hepatic cells**

Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Hepatic DNA damage in rats and mice given single dose MCA, DCA, TCA, or CH
HERO ID:	628837

Domain	Metric		Rating†	MWF*	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified by name as trichloroacetic acid (TCA), dichloroacetic acid (DCA), monochloroacetic acid (MCA), and trichloroacetaldehyde (TCAA). It was reported that TCAA "in water exists as chloral hydrate." The vehicle for TCAA was water.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substances was reported (Sigma).
	Metric 3:	Test Substance Purity	Low	× 1	3	The purities of the test substances were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle control groups were included (water).
	Metric 5:	Positive Controls	High	× 1	1	Concurrent positive controls (N-nitrosodiethylamine and methyl methanesulfonate) were included in the study design and responded as expected
	Metric 6:	Randomized Allocation	Low	× 1	3	The method of allocation of animals to treatment groups was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported. Storage of the test substance was not reported but is unlikely to significantly impact results (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity in Figs 1 and 2 (1, 2, 5, or 10 mmol/kg)
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration (single administration) were reported and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups (2-3) is reasonable. Doses were selected based on lethality, carcinogenicity, or DNA damage in prior studies.

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Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Hepatic DNA damage in rats and mice given single dose MCA, DCA, TCA, or CH
HERO ID:	628837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure (gavage) were appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, commercial source, and starting body weight range of the test animals were reported. The age and initial health status of the test animals were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported, appropriate, and consistent across groups.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per treatment group was slightly lower than recommended for the endpoint (n = 4).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (alkaline unwinding) was appropriate for this endpoint. It was described thoroughly.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcome of interest (3 technical replicates per animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest (determined flourometrically)
	Metric 20:	Negative Control Response	High	× 1	1	Responses in negative controls were reported and appeared to be appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Starting body weight ranges were included. Food and water consumption and respiratory rates were not reported, but this is not expected to significantly impact the results given the short study duration and gavage administration.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The data were analyzed appropriately by Dunnett's multiple comparison test.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data were reported for all groups; however, the variance of the data was not reported (Figure 2).

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Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Hepatic DNA damage in rats and mice given single dose MCA, DCA, TCA, or CH
HERO ID:	628837

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		High		1.4	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 188: **Animal toxicity evaluation results for Chang et al 1992 for DNA damage study in rat hepatic cells**

Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Hepatic DNA damage in rats exposed for 30 wks to DCA
HERO ID:	628837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name as dichloroacetic acid (DCA).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported (Sigma)
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle control group was included (saline).
	Metric 5:	Positive Controls	Low	× 1	3	Although concurrent positive controls were used for this endpoint for other animal experiments within this study, no positive control was used for this chronic rat exposure experiment.
	Metric 6:	Randomized Allocation	Low	× 1	3	The method of allocation of animals to treatment groups was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported. Storage of the test substance was not reported. It was noted that the concentration of test substance in drinking water was confirmed by gas chromatography.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was reported to be consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Doses in drinking water were reported in terms of g/L. No information on water intake was provided; this is likely to have a substantial impact on results.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure duration (30 wks) was reported and appropriate for this endpoint. Frequency is assumed to be daily based on method (drinking water) of administration.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups (3 plus control) and dose spacing were appropriate. The doses were selected based on lethality, carcinogenicity, or DNA damage in prior studies.

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Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Hepatic DNA damage in rats exposed for 30 wks to DCA
HERO ID:	628837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure (drinking water) were appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, commercial source, and starting body weight range of the test animals were reported. The age and initial health status of the test animals were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported, appropriate, and consistent across groups.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and appropriate for this endpoint (n = 5).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (alkaline unwinding) was appropriate for this endpoint. It was described thoroughly.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcome of interest (3 technical replicates per animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest (determined flourometrically)
	Metric 20:	Negative Control Response	High	× 1	1	Responses in negative controls were reported and appeared to be appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Starting body weight ranges were included. Water consumption information was not included, which is a deficiency likely to affect interpretation of results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The data were analyzed appropriately by Dunnett's multiple comparison test.
	Metric 24:	Reporting of Data	High	× 2	2	Results were reported for all treatment groups, including mean, SE, and n.
Overall Quality Determination‡			High		1.6	

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Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Hepatic DNA damage in rats exposed for 30 wks to DCA
HERO ID:	628837

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 189: **Animal toxicity evaluation results for Chang et al 1992 for DNA damage study in mouse hepatic cells**

Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Hepatic DNA damage in mice exposed for 7 or 14 days to DCA
HERO ID:	628837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name as dichloroacetic acid (DCA).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported (Sigma).
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle control group was included (saline).
	Metric 5:	Positive Controls	High	× 1	1	Concurrent positive controls (N-nitrosodiethylamine) were included in the study design.
	Metric 6:	Randomized Allocation	Low	× 1	3	The method of allocation of animals to treatment groups was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported. Storage of the test substance was not reported. It was noted that the concentration of test substance in drinking water was confirmed by gas chromatography.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Doses in drinking water were reported in terms of g/L. No information on water intake was provided; this is likely to have a substantial impact on results.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure duration (7 or 14 days) were reported and appropriate for this endpoint. Frequency is assumed to be daily based on method (drinking water) of administration.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups (2 plus control) and dose spacing (10x) were acceptable but lower than recommended for this endpoint (3 plus control). The doses were selected based on lethality, carcinogenicity, or DNA damage in prior studies. The high dose was sufficient to induce the expected effect.

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Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Hepatic DNA damage in mice exposed for 7 or 14 days to DCA
HERO ID:	628837

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure (drinking water) were appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, commercial source, and starting body weight range of the test animals were reported. The age and initial health status of the test animals were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported, appropriate, and consistent across groups.
	Metric 15:	Number per Group	Low	× 1	3	The number of animals per treatment group was lower than recommended for the endpoint (n = 3).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (alkaline unwinding) was appropriate for this endpoint. It was described thoroughly.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcome of interest (3 technical replicates per animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest (determined flourometrically)
	Metric 20:	Negative Control Response	High	× 1	1	Responses in negative controls were reported and appeared to be appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Starting body weight ranges were included. Water consumption information was not included, which is a deficiency likely to affect interpretation of results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The data were analyzed appropriately by Dunnett's multiple comparison test.
	Metric 24:	Reporting of Data	High	× 2	2	Results were reported for all treatment groups, including mean, SE, and n.
Overall Quality Determination‡			High		1.7	

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Study Citation:	L. W. Chang, F. B. Daniel, A. B. Deangelo (1992). Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes Environmental and Molecular Mutagenesis, 20(4,4), 277-288
Data Type:	Hepatic DNA damage in mice exposed for 7 or 14 days to DCA
HERO ID:	628837

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 190: **Animal toxicity evaluation results for Nelson and Bull 1988 for DNA strand break study**

Study Citation:	M. A. Nelson, R. J. Bull (1988). Induction of strand breaks in DNA by trichloroethylene and metabolites in rat and mouse liver in vivo Toxicology and Applied Pharmacology, 94(1,1), 45-54
Data Type:	in vivo DNA strand breaks
HERO ID:	628935

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE and metabolites were definitively identified by established nomenclature.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial sources (Fisher Scientific and Sigma Chemical) were identified.
	Metric 3:	Test Substance Purity	High	× 1	1	99+% purity
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A vehicle control group was used for each experiment.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required .
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was described (in aqueous Tween). Storage was not described; however, experiments were of short duration (single exposure or 4-5 days).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was consistently administered across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses reported without ambiguity (mmol/kg).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Experiments included single exposure and four or five daily exposures
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	4 groups plus a control used in single exposure experiments; repeat exposure experiments used 2 doses plus control; selected doses produced a range of responses.
	Metric 12:	Exposure Route and Method	High	× 1	1	oral gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, sex, strain, starting body weight and animal source were provided.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Temperature and humidity were not reported; however, rooms were described as "temperature controlled".
	Metric 15:	Number per Group	High	× 1	1	4-7 animals per group

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Study Citation:	M. A. Nelson, R. J. Bull (1988). Induction of strand breaks in DNA by trichloroethylene and metabolites in rat and mouse liver in vivo Toxicology and Applied Pharmacology, 94(1,1), 45-54
Data Type:	in vivo DNA strand breaks
HERO ID:	628935

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	The method (alkaline unwinding) was reported and sensitive for the outcome of of interest.
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18: Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable for the outcome interest.
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable for the outcome interest.
	Metric 20: Negative Control Response	High	× 1	1	Negative controls responded appropriately.
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	Medium	× 2	4	Food and water intake were not reported but unlikely to significantly impact the results
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported; however, this missing information is unlikely to affect the results (exposure duration 4 h). Animals that died from intubation errors were excluded.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate (Student's t test for difference of slopes; ANOVA and Duncan's multiple range test for differences among groups).
	Metric 24: Reporting of Data	High	× 2	2	Data were presented (mean, SE, n) for all outcomes by exposure group. Control groups were pooled for presentation of results from repeated exposure experiments.
Overall Quality Determination‡		High		1.3	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 191: **Animal toxicity evaluation results for Toraason et al 1999 for acute study in rats on DNA damage**

Study Citation:	M. Toraason, J. Clark, D. Dankovic, P. Mathias, S. Skaggs, C. Walker, D. Werren (1999). Oxidative stress and DNA damage in Fischer rats following acute exposure to trichloroethylene or perchloroethylene Toxicology, 138(1,1), 43-53
Data Type:	DNA damage for TCE (8OHdG adducts)
HERO ID:	628948

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as trichloroethylene (TCE).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be 99.9% pure (HPLC grade).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were treated with a 1:4 v/v ratio of Alkamuls® to water.
	Metric 5:	Positive Controls	High	× 1	1	Concurrent positive controls were treated with 2-nitropropane in vehicle. Positive controls responded appropriately.
	Metric 6:	Randomized Allocation	High	× 1	1	It was reported that animals were randomly allocated into the treatment groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported. Test substance storage was not reported, but this is appropriate given the study design (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure parameters were consistent among treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency (single-dose administration) and duration (12 hr and 24 hr urine sample collection; 24 hr sacrifice) were reported and appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose spacing was reported and appropriate. It should be noted that only the mid-dose (500 mg/kg) was tested for liver and lymphocyte 8OHdG due to cost restraints. However, this dose to be tested for these endpoints was selected based on the highest TBARS values (oxidative stress). Furthermore, both health effects and a positive response were observed for liver 8OHdG at this dose, indicating that the dose was adequate.

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Study Citation:	M. Toraason, J. Clark, D. Dankovic, P. Mathias, S. Skaggs, C. Walker, D. Werren (1999). Oxidative stress and DNA damage in Fischer rats following acute exposure to trichloroethylene or perchloroethylene Toxicology, 138(1,1), 43-53
Data Type:	DNA damage for TCE (8OHdG adducts)
HERO ID:	628948

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route was reported and appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species, strain, sex, and starting body weight range were reported. Test animal health status and age were not reported, but this is not expected to have substantially impacted results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	It was reported that rats were housed individually, but no details regarding temperature, humidity, or light-dark cycles were reported.
	Metric 15:	Number per Group	High	× 1	1	Each treatment group consisted of n = 6 rats.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for the endpoint of interest (DNA damage in liver and lymphocytes). The detection of 8OHdG in urine via HPLC-EC was considered exploratory and was not assessed for this review.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	Low	× 1	3	It was unclear how many technical replicates per animal were included in the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 20:	Negative Control Response	High	× 1	1	The negative controls responded appropriately.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food and water intake were not reported for each group.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Data were appropriately analyzed by ANOVA; however, it was not specified whether a one-way or two-way ANOVA was used, and the post-hoc test was not specified.
	Metric 24:	Reporting of Data	Medium	× 2	4	Only one of three dose levels were tested for the liver and lymphocyte 8OHdG endpoint.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

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Study Citation:	M. Toraason, J. Clark, D. Dankovic, P. Mathias, S. Skaggs, C. Walker, D. Werren (1999). Oxidative stress and DNA damage in Fischer rats following acute exposure to trichloroethylene or perchloroethylene Toxicology, 138(1,1), 43-53
Data Type:	DNA damage for TCE (8OHdG adducts)
HERO ID:	628948

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 192: **Animal toxicity evaluation results for Russo et al 1984 for mouse spermatid aneuploidy study**

Study Citation:	A. Russo, F. Pacchierotti, P. Metalli (1984). Nondisjunction induced in mouse spermatogenesis by chloral hydrate, a metabolite of trichloroethylene Environmental Mutagenesis, 6(5,5), 695-703
Data Type:	in vivo i.p. mouse sperm aneuploidy chloral hydrate
HERO ID:	630935

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as Chloral Hydrate (CH)
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance and product number was identified. The material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was reported (99 %)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative controls were used. It was reported that untreated mice served as controls; there were no vehicle (distilled water) or sham-treated controls, but this is not expected to significantly impact the aneuploidy results
	Metric 5:	Positive Controls	Not Rated	NA	NA	The use of positive controls was not applicable for this study.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was dissolved in distilled water (not further described). Storage of the test substance was not reported but is unlikely to impact results because substance administered as a single i.p. injection.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Exposure administration was consistent across treatment groups , except that controls were not sham-treated.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported as mg/kg bw without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for this endpoint; single exposure.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose spacing were justified by the study authors based on previous published studies.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route was appropriate for the test substance.
Domain 4: Test Organism						

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Study Citation:	A. Russo, F. Pacchierotti, P. Metalli (1984). Nondisjunction induced in mouse spermatogenesis by chloral hydrate, a metabolite of trichloroethylene Environmental Mutagenesis, 6(5,5), 695-703
Data Type:	in vivo i.p. mouse sperm aneuploidy chloral hydrate
HERO ID:	630935

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The test animal species, strain, sex, and age were reported while health status and starting body weight were not. The source of the test animals was not reported. The test species and strain were an appropriate animal model for the evaluation of this endpoint.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and if differences occurred between control and exposed populations.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group (6/dose/time point) was reported and appropriate for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodologies were appropriate for the endpoints of interest. Some methodology details were reported in a previous study.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest (600 cells from 6 mice/group/stage were analyzed).
	Metric 19:	Blinding of Assessors	High	× 1	1	Slides were coded for scoring
	Metric 20:	Negative Control Response	Medium	× 1	2	The biological responses of the negative control groups were reported; however, there were minor limitations regarding the control responses. One control animal had an unusually high index of hyperhaploidy making the group show a significant heterogeneity; this mouse was not included in the estimate of the spontaneous frequency of nondisjunction. The remaining control group consisted of 16 untreated animals.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weights were not reported. Food and water consumption were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical method (chi squared) were described and appropriate for the dataset.

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Study Citation:	A. Russo, F. Pacchierotti, P. Metalli (1984). Nondisjunction induced in mouse spermatogenesis by chloral hydrate, a metabolite of trichloroethylene Environmental Mutagenesis, 6(5,5), 695-703
Data Type:	in vivo i.p. mouse sperm aneuploidy chloral hydrate
HERO ID:	630935

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	High	× 2	2	Data were reported for all exposure groups, including mean, SEM, and n.
Overall Quality Determination‡		Medium		1.7	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 193: **Animal toxicity evaluation results for Mally et al 2006 for tumor mutation analysis study**

Study Citation:	A. Mally, C. Walker, J. Everitt, W. Dekant, S. Vamvakas (2006). Analysis of renal cell transformation following exposure to trichloroethene in vivo and its metabolite S-(dichlorovinyl)-L-cysteine in vitro Toxicology, 224(1-2,1-2), 108-118					
Data Type:	Mutation analysis of tumors (Tsc-2 and VHL)					
HERO ID:	700373					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was reported as trichloroethene
	Metric 2:	Test Substance Source	High	× 1	1	Reported purchased from Sigma-aldrich unless otherwise indicated
	Metric 3:	Test Substance Purity	High	× 1	1	TCE purity reported > 99.5%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative vehicle controls (corn oil only) were reported
	Metric 5:	Positive Controls	Not Rated	NA	NA	NA: positive control was not necessary based on study type
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance in corn oil were reported. Storage conditions were not reported
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Exposure administration was consistent across groups: daily (5d/wk) by gavage with equal volumes (not reported) that were assumed to be appropriate for the species. The time of day was not reported but this is not likely to significantly impact the results.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported 50, 100, 250, 500 and 1000 mg/kg bw
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure administration was 5d/wk for 13 wks and adequate for the study type
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The justification for number and spacing of doses was not reported. 5 dose groups plus control were used. None of the doses induced a significant change from control with respect to preneoplastic lesions or tumor incidences so it is not clear that the high dose was high enough.
	Metric 12:	Exposure Route and Method	High	× 1	1	Route (oral gavage) is appropriate for the study type
Domain 4: Test Organism						

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Study Citation:	A. Mally, C. Walker, J. Everitt, W. Dekant, S. Vamvakas (2006). Analysis of renal cell transformation following exposure to trichloroethene in vivo and its metabolite S-(dichlorovinyl)-L-cysteine in vitro Toxicology, 224(1-2,1-2), 108-118
Data Type:	Mutation analysis of tumors (Tsc-2 and VHL)
HERO ID:	700373

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain and sex were reported and source was described in detail. The strain (Eker Tsc-2EK/+) is a specialized strain carrying mutation in the tuberous sclerosis tumor suppressor gene. Relevance of the model is uncertain.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported , consistent across groups, and adequate for the study type
	Metric 15:	Number per Group	High	× 1	1	Group sizes between 11 and 21 were used.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment (mutation analysis by RT or multiplex PCR) methodology was described and appropriate for the endpoint
	Metric 17:	Consistency of Outcome Assessment		× 1	NA	The outcome assessment was consistent in protocol and time across all study groups
	Metric 18:	Sampling Adequacy	Unacceptable	× 1	4	1-4 tumors from each treatment group were analyzed for loss of homozygosity in Tsc-2 genotype and VHL gene mutation, but there were 7-11 tumors per group . This is a serious flaw that makes the study unusable
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for this study type
	Metric 20:	Negative Control Response	Medium	× 1	2	The biological response in the negative control group was reported but only 2 of 7 tumors analyzed.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weight and food and water intake were not reported but are unlikely to have a significant impact on the mutation analysis
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	Not applicable for the study type
	Metric 24:	Reporting of Data	High	× 2	2	Results were reported for all tumors sampled in table 4
Overall Quality Determination‡			Unacceptable⋆⋆		0.0	
Extracted			Yes			

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Study Citation:	A. Mally, C. Walker, J. Everitt, W. Dekant, S. Vamvakas (2006). Analysis of renal cell transformation following exposure to trichloroethene in vivo and its metabolite S-(dichlorovinyl)-L-cysteine in vitro Toxicology, 224(1-2,1-2), 108-118
Data Type:	Mutation analysis of tumors (Tsc-2 and VHL)
HERO ID:	700373

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 194: **Animal toxicity evaluation results for Beland 1999 for mouse bone marrow micronucleus study**

Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Mouse bone marrow micronucleus for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was 99.5%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (phosphate-buffered saline i.p. injection).
	Metric 5:	Positive Controls	High	× 1	1	Appropriate concurrent positive control groups were included (cyclophosphamide injection).
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	Method of allocation of animals to treatment groups was not reported for the in vivo bone marrow micronucleus assay, although other experiments used random allocation. More detailed methods were cited to other references.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The preparation and storage of the test substance were thoroughly described.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity in mg/kg bw.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration were reported and appropriate for this endpoint (3 injections at 24-hr intervals).
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose spacing were selected based on range finding studies
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure (i.p. injection) were appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species and sex of the test animals was reported. The strain, age, commercial source, and starting body weight range were not reported for the in vivo micronucleus study. However, more detailed methods were cited to other references.

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Mouse bone marrow micronucleus for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	The husbandry conditions were not reported explicitly for the micronucleus test, but conditions for other animal test were reported and appropriate. More detailed micronucleus assay methods are cited to other references.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per treatment group was adequate and appropriate for these endpoints (n = 4-5).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for this endpoint.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest (2,000 polychromatic erythrocytes evaluated per animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to this study type. Detailed methodology for this assay was cited to another reference.
	Metric 20:	Negative Control Response	High	× 1	1	Responses in negative controls were appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weight ranges were not reported for this study.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No attrition or health outcomes unrelated to exposure were reported in any treatment group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Data were appropriately analyzed by one-tailed trend test.
	Metric 24:	Reporting of Data	High	× 2	2	All data were reported adequately (mean , SD, and n).
Overall Quality Determination[‡]			High		1.2	
Extracted			Yes			

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Mouse bone marrow micronucleus for chloral hydrate
HERO ID:	701161

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 195: **Animal toxicity evaluation results for Douglas et al 1999 for mutagenicity study**

Study Citation:	G. Douglas, J. Gingerich, L. Soper, M. Potvin, S. Bjarnason (1999). Evidence for the lack of base-change and small-deletion mutation induction by trichloroethylene in lacZ transgenic mice Environmental and Molecular Mutagenesis, 34(2-3,2-3), 190-194
Data Type:	Mutation in lacZ transgenic mice
HERO ID:	701798

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by established nomenclature.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified. Batch/lot number was not provided, but the test material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	99+%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	A negative control was indicated by the 0 ppm group; however, sham-treatment was not explicitly described.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The methods and equipment used to generate the test atmosphere were described and appropriate (glass evaporative system).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and consistent across groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Target and measured concentrations were reported. Range of concentrations was >10% target concentration for two of the three doses. Analytical method was reported and appropriate (GC).
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	Exposure frequency and duration was reported and appropriate (6h/day for 12 days). Negative findings; therefore, difficult to know whether the exposure duration was sufficient in the absence of reported health effects.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	3 treatment groups plus control (greater than 10-fold difference from lowest to highest concentration). Negative findings; therefore, difficult to know whether highest concentration was high enough in the absence of reported health effects.

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Study Citation:	G. Douglas, J. Gingerich, L. Soper, M. Potvin, S. Bjarnason (1999). Evidence for the lack of base-change and small-deletion mutation induction by trichloroethylene in lacZ transgenic mice Environmental and Molecular Mutagenesis, 34(2-3,2-3), 190-194
Data Type:	Mutation in lacZ transgenic mice
HERO ID:	701798

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Dynamic whole-body chamber; TCE not expected to condense. ~10 chamber volumes/hr.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Details regarding the transgenic mice were provided in another study (Gossen et al., 1989). However, the details provided indicated that this mouse strain is appropriate for the outcome of interest.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Temperature and humidity were reported for chamber air. Light-dark cycle was not reported.
	Metric 15:	Number per Group	High	× 1	1	5-10/sex/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Sampling times of 14 days and 60 days. Mutations fixed after 14 days may be repaired or cleared via cell death/turnover.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome.
	Metric 20:	Negative Control Response	High	× 1	1	The response in the negative control group seemed appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food/water intake, and respiratory rate were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported. This is a substantial deficiency, as the presence or absence of health effects helps to determine whether the highest dose was high enough, or whether the exposure time was sufficient to detect a positive response.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	No statistical analyses were performed but sufficient data were provided to conduct an independent statistical analysis (mean +/- SEM for mutant frequency).
	Metric 24:	Reporting of Data	Medium	× 2	4	60-day data were fully reported. 14-day data were only reported qualitatively for some organs or left unreported.
Overall Quality Determination‡			Medium		1.9	

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Study Citation:	G. Douglas, J. Gingerich, L. Soper, M. Potvin, S. Bjarnason (1999). Evidence for the lack of base-change and small-deletion mutation induction by trichloroethylene in lacZ transgenic mice Environmental and Molecular Mutagenesis, 34(2-3,2-3), 190-194
Data Type:	Mutation in lacZ transgenic mice
HERO ID:	701798

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 196: **Animal toxicity evaluation results for Giller et al 1995 for micronucleus study**

Study Citation:	S. Giller, F. Le Curieux, L. Gauthier, F. Erb, D. Marzin (1995). Genotoxicity assay of chloral hydrate and chloropicrine Mutation Research, 348(4,4), 147-152
Data Type:	Chloral hydrate in vivo MN pleurodeles waltl larvae
HERO ID:	702123

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chloral hydrate identified by name, molecular formula, and CASRN (CI3CCH(OH)2, CAS 302-17-0)
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source reported: Aldrich (St Quentin Falavier, France)
	Metric 3:	Test Substance Purity	High	× 1	1	99% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	A negative control was reported in table 2 but it is unclear if it is an untreated or solvent control
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for the study type
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	Allocation of animals into study groups is not reported but may have been described in test protocols cited to other publications
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Preparation of the test substance was not reported (no information on vehicle)
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	Exposure methods were cited to another publication with no additional details.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported as 0, 50, 100, 200, 400 ug/ml in table 2
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	Exposure duration (12d) was reported and is appropriate for the study type. Frequency was not reported in text but is assumed to be continuous (larval exposure) and may have been detailed in methods publications cited.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Number of dose groups was reported. Justification of the doses and spacing was not provided.
	Metric 12:	Exposure Route and Method	Not Rated	NA	NA	Exposure route is not reported but may be reported in methods papers cited
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Test animal species was reported (newt pleurodeles waltl larvae) but is not commonly used. The source of the test species was not reported . The test animal is not commonly used

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Study Citation:	S. Giller, F. Le Curieux, L. Gauthier, F. Erb, D. Marzin (1995). Genotoxicity assay of chloral hydrate and chloropicrine Mutation Research, 348(4,4), 147-152
Data Type:	Chloral hydrate in vivo MN pleurodeles waltl larvae
HERO ID:	702123

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were not reported
	Metric 15:	Number per Group	Not Rated	NA	NA	Number of animals per group was not reported but may have been cited previously
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Outcome assessment was not reported but may be reported in cited methods publications
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Outcome assessment was not reported but may be reported in cited methods publications
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Outcome assessment was not reported but may be reported in cited methods publications
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Outcome assessment was not reported but may be reported in cited methods publications
	Metric 20:	Negative Control Response	High	× 1	1	The biological response in the negative control group was reported and appeared to be adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Conditions that could influence outcome assessment were not reported
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical methods were cited to another publication; data reported are not sufficient for independent statistical analysis because n/group is not reported
	Metric 24:	Reporting of Data	Low	× 2	6	Data reported for all dose groups (mean and SD; n/group not reported)
Overall Quality Determination‡			Unacceptable⋆⋆		2.3	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 197: **Animal toxicity evaluation results for Grawe et al 1997 for micronucleus study**

Study Citation:	J. Grawé, M. Nüsse, I. D. Adler (1997). Quantitative and qualitative studies of micronucleus induction in mouse erythrocytes using flow cytometry: I: Measurement of micronucleus induction in peripheral blood polychromatic erythrocytes by chemicals with known and suspected genotoxicity Mutagenesis, 12(1,1), 1-8
Data Type:	In vivo i.p. micronucleus assay - Chloral Hydrate metabolite
HERO ID:	702190

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as Chloral Hydrate (CH) with appropriate CAS number
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified, though the manufacturer and batch numbers were not. The material is not expected to vary in composition
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Treatment groups were compared within themselves at exposure time "zero" and later sampling times.
	Metric 5:	Positive Controls	Not Rated	NA	NA	The use of positive controls was not applicable for this study type
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance was dissolved in didistilled water (not further described). Storage of the test substance was not reported but is unlikely to significantly impact results because administration was a single i.p. injection.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (mg/kg bw).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were reported and appropriate for this endpoint; single i.p. dose
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only one dose was tested; the dose was chosen based on previous study showing positive result.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The exposure route (i.p. injection) is acceptable for the test substance but not recommended for the outcome of interest without specific justification (not provided in study).
Domain 4: Test Organism						

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Study Citation:	J. Grawé, M. Nüsse, I. D. Adler (1997). Quantitative and qualitative studies of micronucleus induction in mouse erythrocytes using flow cytometry: I: Measurement of micronucleus induction in peripheral blood polychromatic erythrocytes by chemicals with known and suspected genotoxicity Mutagenesis, 12(1,1), 1-8
Data Type:	In vivo i.p. micronucleus assay - Chloral Hydrate metabolite
HERO ID:	702190

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, age, starting body weight, and source were reported for the main mouse strain, while health status was not. The test species and strains were an appropriate animal model for the evaluation of this endpoint. A second strain was tested for comparison with previous results; details for this strain were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per study group was reported (3/group) and lower than recommended (5) for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were reported in detail and appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across experiments and treatment groups
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was robust for the outcomes of interest (~45,000 PCEs).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for this study; automated assessments
	Metric 20:	Negative Control Response	Medium	× 1	2	The biological responses of the test groups at time zero were adequate. As a result of the large numbers of PCE evaluated, small differences in time zero MN frequencies across experiments with different compounds were shown to be significant.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weights were not reported. Food and water consumption were not reported, but this is not expected to significantly impact the results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were described and appropriate for the dataset.

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Study Citation:	J. Grawé, M. Nüsse, I. D. Adler (1997). Quantitative and qualitative studies of micronucleus induction in mouse erythrocytes using flow cytometry: I: Measurement of micronucleus induction in peripheral blood polychromatic erythrocytes by chemicals with known and suspected genotoxicity Mutagenesis, 12(1,1), 1-8
Data Type:	In vivo i.p. micronucleus assay - Chloral Hydrate metabolite
HERO ID:	702190

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	Medium	× 2	4	Both individual animal data and summary data (means and SDs) were reported. Numbers of PCE evaluated per animal were reported semi-quantitatively (~45000).
Overall Quality Determination‡		Medium		1.8	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 198: **Animal toxicity evaluation results for Jaffe et al 1985 for renal DNA damage study**

Study Citation:	D. Jaffe, C. Hassall, A. Gandolfi, K. Brendel (1985). Production of DNA single strand breaks in rabbit renal tissue after exposure to 1,2-dichlorovinylcysteine Toxicology, 35(1,1), 25-33
Data Type:	DCVC rabbit renal dna damage
HERO ID:	704496

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	1,2-dichlorovinylcysteine (DCVC) identified by name
	Metric 2:	Test Substance Source	Medium	× 1	2	DCVC was synthesized; methods cited to another publication
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative controls are reported in tables but it is unclear if they are untreated or solvent controls
	Metric 5:	Positive Controls	Not Rated	NA	NA	not applicable for the study type
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported; storage conditions were not reported but not expected to influence results (single exposure)
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Time of day when exposure occurred was not reported but is unlikely to have a substantial impact on results.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses (10, 20, 50, 100 mg/kg bw) are reported unambigously
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration (single injection) was reported and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Number of exposure groups is appropriate for the study type (control plus 2 or 3 depending on route). The highest i.v. dose was limited by solubility.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The routes of exposure (i.v. and i.p.) were acceptable but not preferred this endpoint.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal characteristics (species, strain, sex, age, and body weight) were reported. Initial health status was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported (lacked information on temperature, humidity, light/dark cycle)

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Study Citation:	D. Jaffe, C. Hassall, A. Gandolfi, K. Brendel (1985). Production of DNA single strand breaks in rabbit renal tissue after exposure to 1,2-dichlorovinylcysteine Toxicology, 35(1,1), 25-33
Data Type:	DCVC rabbit renal dna damage
HERO ID:	704496

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Low	× 1	3	The number of animals was lower than recommended (n=2 from table 1; 5 recommended)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (alkaline elution) was fully described and appropriate for this endpoint.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent for all groups.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Number of cells evaluated per animal not reported.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the study type
	Metric 20:	Negative Control Response	High	× 1	1	The negative control response was reported and appeared adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Food/water intake were not reported but are unlikely to significantly impact results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	Statistical analysis was not performed for in vivo studies and data reported were not sufficient to allow an independent statistical analysis (only mean reported, no measure of variability)
	Metric 24:	Reporting of Data	Low	× 2	6	Data were reported for all exposure groups as mean elution rate. Data reported were not sufficient to allow an independent statistical analysis (no measure of variability)
Overall Quality Determination‡			Unacceptable⋆⋆		2.0	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 199: **Animal toxicity evaluation results for Leopardi et al 1993 for acute study in mice on micronuclei**

Study Citation:	P. Leopardi, A. Zijno, B. Bassani, F. Pacchierotti (1993). In vivo studies on chemically induced aneuploidy in mouse somatic and germinal cells Mutation Research, 287(1,1), 119-130
Data Type:	Micronuclei for chloral hydrate
HERO ID:	706726

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate by name and CASRN.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was identified as a private donor, not a commercial source. Analytical verification of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance is not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were used (water vehicle).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Standard positive controls such as mitomycin C were not included; however, positive responses were observed from other test substances in this study, so this is not considered to have had a substantial impact on results.
	Metric 6:	Randomized Allocation	Low	× 1	3	The method of allocation of animals to experimental groups was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The preparation of the test substance was briefly reported. Storage of the test substance was not reported, but this is not expected to significantly impact results given the duration (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across exposure groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported (mg/kg bw) without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for this endpoint (single dose; sample collection at 18 and 24 hr post-injection).
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Two exposure groups plus control were used. Doses were justified based on published data on c-mitotic effects in bone marrow. Dose spacing was sufficient to show a range of responses.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route (i.p. injection) was reported and appropriate for the test substance.
Domain 4: Test Organism						

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Study Citation:	P. Leopardi, A. Zijno, B. Bassani, F. Pacchierotti (1993). In vivo studies on chemically induced aneuploidy in mouse somatic and germinal cells Mutation Research, 287(1,1), 119-130
Data Type:	Micronuclei for chloral hydrate
HERO ID:	706726

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The test animal species, strain, sex, and age were reported. The test animal starting body weight range and commercial source were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per group was slightly lower than typical for these endpoints (n = 4/dose and time point).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was described and appropriate for the endpoint of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Sampling was low for the outcome of interest (1,000 polychromatic erythrocytes per mouse; guidance recommends 4,000).
	Metric 19:	Blinding of Assessors	Low	× 1	3	Study did not report coding of slides. It is not clear whether slides were scored manually or using automation.
	Metric 20:	Negative Control Response	High	× 1	1	Responses in negative control groups were reported and appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food and water consumption were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No attrition or health outcomes unrelated to exposure were reported for CH.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Data were appropriately analyzed by Mann-Whitney U-test.
	Metric 24:	Reporting of Data	High	× 2	2	Results were reported for all treatment groups including mean, SE, and n
Overall Quality Determination‡			Medium		1.8	
Extracted			Yes			

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Study Citation:	P. Leopardi, A. Zijno, B. Bassani, F. Pacchierotti (1993). In vivo studies on chemically induced aneuploidy in mouse somatic and germinal cells Mutation Research, 287(1,1), 119-130
Data Type:	Micronuclei for chloral hydrate
HERO ID:	706726

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 200: **Animal toxicity evaluation results for Leopardi et al 1993 for acute study in mice on hyperploidy**

Study Citation:	P. Leopardi, A. Zijno, B. Bassani, F. Pacchierotti (1993). In vivo studies on chemically induced aneuploidy in mouse somatic and germinal cells Mutation Research, 287(1,1), 119-130					
Data Type:	Hyperploidy for chloral hydrate					
HERO ID:	706726					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as chloral hydrate by name and CASRN.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was identified as a private donor, not a commercial source. Analytical verification of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were used (water vehicle).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not included; however, positive responses were observed from other test substances in this study, so this is not considered to have had a substantial impact on results.
	Metric 6:	Randomized Allocation	Low	× 1	3	The method of allocation of animals to experimental groups was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance preparation was briefly described. The storage of the test substance was not reported, but this is not expected to significantly impact results given the duration (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported (mg/kg bw) without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for this endpoint (single dose).
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Two exposure groups plus control were used. Doses were justified based on published data on c-mitotic effects in bone marrow. Dose spacing was sufficient to show a range of responses.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route (i.p. injection) was reported and appropriate for the test substance.
Domain 4: Test Organism						

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Study Citation:	P. Leopardi, A. Zijno, B. Bassani, F. Pacchierotti (1993). In vivo studies on chemically induced aneuploidy in mouse somatic and germinal cells Mutation Research, 287(1,1), 119-130
Data Type:	Hyperploidy for chloral hydrate
HERO ID:	706726

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The test animal species, strain, sex, and age were reported. The test animal starting body weight range and commercial source were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per group was slightly lower than typical for these endpoints (n = 4/dose and time point).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology was briefly described and appropriate for the endpoint of interest. Preparation and staining of bone marrow metaphases was cited to other publications.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Sampling was somewhat low for the outcome of interest (100 second metaphases per mouse) based on comparison to guidance for CAs (400 metaphases per mouse); guidance for aneuploidy assessment is not available
	Metric 19:	Blinding of Assessors	Low	× 1	3	Study did not report coding of slides. It is not clear whether slides were scored manually or using automation.
	Metric 20:	Negative Control Response	High	× 1	1	Responses in negative control groups were reported and appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food and water consumption were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No attrition or health outcomes unrelated to exposure were reported for CH.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The data were analyzed appropriately by G-test.
	Metric 24:	Reporting of Data	High	× 2	2	Results were reported for all treatment groups including mean, SE, and n
Overall Quality Determination‡			Medium		1.8	
Extracted			Yes			

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Study Citation:	P. Leopardi, A. Zijno, B. Bassani, F. Pacchierotti (1993). In vivo studies on chemically induced aneuploidy in mouse somatic and germinal cells Mutation Research, 287(1,1), 119-130
Data Type:	Hyperploidy for chloral hydrate
HERO ID:	706726

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 201: **Animal toxicity evaluation results for Leuschner and Leuschner 1991 for rat study on chromosomal aberrations**

Study Citation:	J. Leuschner, F. Leuschner (1991). Evaluation of the mutagenicity of chloral hydrate in vitro and in vivo Arzneimittel-Forschung, 41(10,10), 1101-1103
Data Type:	Rat chromosomal aberrations
HERO ID:	706734

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance (chloral hydrate) was identified by established nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer was reported. Batch/lot number was not given, but the composition is not expected to vary.
	Metric 3:	Test Substance Purity	High	× 1	1	>99.4%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative vehicle controls were used.
	Metric 5:	Positive Controls	High	× 1	1	Cyclophosphamide was used as the positive control and a positive response was observed.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance was prepared in 0.9% NaCl. Storage conditions were not described; however, a single i.p. dose was used suggesting that this is not likely to have a substantial impact on the results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Same gavage volume (20 ml/kg bw) was administered for each group.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity as mg/kg bw.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Bone marrow harvested at 6, 24 and 48h.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Three doses plus control. The high dose was lethal to 4/30 rats; clinical signs were observed at this dose (ataxia, reduced motility, reduced muscular tonus, dyspnea, myosis, abdominal position) and some at lower doses
	Metric 12:	Exposure Route and Method	High	× 1	1	Oral gavage is an acceptable route.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Source, species, strain sex and starting body weight were provided. Age and health status were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.

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Study Citation:	J. Leuschner, F. Leuschner (1991). Evaluation of the mutagenicity of chloral hydrate in vitro and in vivo Arzneimittel-Forschung, 41(10,10), 1101-1103
Data Type:	Rat chromosomal aberrations
HERO ID:	706734

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Low	× 1	3	5/sex/dose/sampling time was assumed based on a) guidelines; b) numbers used for mouse micronucleus study in same paper and 3) 4/30 died in the high dose group and there were 3 sampling times. Number/group was not indicated in the methods or results.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The method was well described and sensitive for the outcome. An EC test guideline was referenced.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Aberrations were assessed in 50 cells/animal or 500 metaphases/group. OECD Guidance suggests that 200 metaphases per animal should be analyzed.
	Metric 19:	Blinding of Assessors	High	× 1	1	Slides were randomised and coded prior to scoring
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were reported and adequate (compared to the positive controls).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The lack of reporting of body weight and food/water intake is not likely to have a significant impact on the results (i.p. injection study).
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Mortality was reported in the highest dose group (considered MTD). Clinical signs of toxicity were reported in all dose groups but the nature and frequencies were not given.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistics were performed, but the method was not specified. Data provided were not sufficient (lacked variability an n/group) for independent statistical analysis.
	Metric 24:	Reporting of Data	Low	× 2	6	Data were reported for each exposure group but lacked a measure of variability and n/group
Overall Quality Determination‡			Medium		1.7	
Extracted			Yes			

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Study Citation:	J. Leuschner, F. Leuschner (1991). Evaluation of the mutagenicity of chloral hydrate in vitro and in vivo Arzneimittel-Forschung, 41(10,10), 1101-1103
Data Type:	Rat chromosomal aberrations
HERO ID:	706734

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 202: **Animal toxicity evaluation results for Leuschner and Leuschner 1991 for rat study on micronuclei**

Study Citation:	J. Leuschner, F. Leuschner (1991). Evaluation of the mutagenicity of chloral hydrate in vitro and in vivo Arzneimittel-Forschung, 41(10,10), 1101-1103
Data Type:	Mouse micronucleus assay
HERO ID:	706734

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance (chloral hydrate) was identified by established nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer was reported. Batch/lot number was not given, but the composition is not expected to vary.
	Metric 3:	Test Substance Purity	High	× 1	1	>99.4%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative vehicle controls were used.
	Metric 5:	Positive Controls		× 1	NA	Cyclophosphamide was used as the positive control and a positive response was observed.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance was prepared in 0.9% NaCl. Storage conditions were not described; however, a single i.p. dose was used suggesting that this is not likely to have a substantial impact on the results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Same injection volume (10 ml/kg bw) was administered for each group.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity in mg/kg bw.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Single dose (2 or more preferred). Bone marrow harvested at 24, 48 and 72h.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single dose group (500 mg/kg). This dose was within the range of the MTD based on clinical signs observed (sedation for 6 hr, ataxia, piloerection)
	Metric 12:	Exposure Route and Method	Low	× 1	3	Intraperitoneal injection is acceptable but not the preferred route for the mouse micronucleus assay.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Source, species, strain sex and starting body weight were provided. Age and health status were not reported.

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Study Citation:	J. Leuschner, F. Leuschner (1991). Evaluation of the mutagenicity of chloral hydrate in vitro and in vivo Arzneimittel-Forschung, 41(10,10), 1101-1103
Data Type:	Mouse micronucleus assay
HERO ID:	706734

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	High	× 1	1	5/sex/sampling time
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The method was well described (with reference to another publication for smear preparation) and sensitive for the outcome. An EC test guideline was referenced.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Micronuclei were assessed in 1000 cells/group (2000 cells is recommeded).
	Metric 19:	Blinding of Assessors	High	× 1	1	Slides were randomised and coded prior to examination
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were reported and adequate (compared to the positive controls).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The lack of reporting of data on body weight and food/water intake is not likely to have a significant impact on the results (i.p. injection study).
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistics were described and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for each exposure group (mean+/-SEM).
Overall Quality Determination‡			High		0.0	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 203: **Animal toxicity evaluation results for Mailhes et al 1993 for mouse oocyte cytogenicity study**

Study Citation:	J. Mailhes, M. Aardema, F. Marchetti (1993). Investigation of aneuploidy induction in mouse oocytes following exposure to vinblastine-sulfate, pyrimethamine, diethylstilbestrol diphosphate, or chloral hydrate Environmental and Molecular Mutagenesis, 22(2,2), 107-114
Data Type:	in vivo i.p. cytogenic assay in mouse oocytes - CH
HERO ID:	706863

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as Chloral Hydrate (CH) with appropriate CAS number
	Metric 2:	Test Substance Source	High	× 1	1	The source (Sigma Chemical) of the test substance was identified, batch/lot number was not. The material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were given i.p injection of the solvent (distilled water)
	Metric 5:	Positive Controls	Not Rated	NA	NA	The use of positive controls was not applicable for this study. On of the other chemicals tested yielded positive results.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation was reported; the solutions were made 2 hours before administration and did not need to be stored.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (mg/kg bw).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for this endpoint; single i.p. dose
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups and spacing of exposure levels were not explicitly justified, although authors noted that the work was an extension of previous studies using lower doses. It is not clear that the high dose was high enough.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route (i.p.) was appropriate for the test substance
Domain 4: Test Organism						

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Study Citation:	J. Mailhes, M. Aardema, F. Marchetti (1993). Investigation of aneuploidy induction in mouse oocytes following exposure to vinblastine-sulfate, pyrimethamine, diethylstilbestrol diphosphate, or chloral hydrate Environmental and Molecular Mutagenesis, 22(2,2), 107-114
Data Type:	in vivo i.p. cytogenic assay in mouse oocytes - CH
HERO ID:	706863

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, age, and starting body weight were reported while health status was not. The test animal was from a reported commercial source. The test species and strain were an appropriate animal model for the evaluation of this endpoint. The uncertainties in reporting are unlikely to have a substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were sufficiently reported.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per study group was reported and appropriate for the study type and outcome analysis; 30 mice/treatment group with the exception of the highest dose (n=15); there was no explanation of why the highest dose group had half the number of mice. Controls (n=35).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across dose groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest (> 1000 oocytes/mouse) with the exception of the high dose group (626 oocytes).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for this study
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the control was reported and appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Food and water consumption were not reported, but this is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were described (Chi square and Fisher's Exact) and appropriate for the dataset.
	Metric 24:	Reporting of Data	Medium	× 2	4	Results were reported for all groups but without a measure of variability
Overall Quality Determination‡			High		1.5	

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Study Citation:	J. Mailhes, M. Aardema, F. Marchetti (1993). Investigation of aneuploidy induction in mouse oocytes following exposure to vinblastine-sulfate, pyrimethamine, diethylstilbestrol diphosphate, or chloral hydrate Environmental and Molecular Mutagenesis, 22(2,2), 107-114
Data Type:	in vivo i.p. cytogenic assay in mouse oocytes - CH
HERO ID:	706863

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 204: **Animal toxicity evaluation results for Nutley et al 1996 for mouse spermatid micronuclei study**

Study Citation:	E. Nutley, A. Tcheong, J. Allen, B. Collins, M. Ma, X. Lowe, J. Bishop, D. Moore, A. Wyrobek (1996). Micronuclei induced in round spermatids of mice after stem-cell treatment with chloral hydrate: evaluations with centromeric DNA probes and kinetochore antibodies Environmental and Molecular Mutagenesis, 28(2,2), 80-89
Data Type:	Micronuclei in round spermatids of mice
HERO ID:	707248

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chloral hydrate identified by established nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Source was NTP.
	Metric 3:	Test Substance Purity	High	× 1	1	97% purity confirmed by capillary GC
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Study authors acknowledged using a concurrent negative control group, but details regarding the negative control group were not reported.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the outcome.
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly allocated to groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported. Storage was not described, but is unlikely to have an impact on results (single i.p. injection).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was consistently administered across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (mg/kg bw).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single dose; 3 timepoints for evaluation.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Doses were not justified; however, a dose-response relationship was apparant for some experiments (3 groups plus a control).
	Metric 12:	Exposure Route and Method	Low	× 1	3	I.p. injection is acceptable but not recommended for this assay.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex and starting age were reported. Health status and starting body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most husbandry conditions were reported (light/dark cycle and temperature). Humidity was not reported.
	Metric 15:	Number per Group	High	× 1	1	4-6/group/time point
Domain 5: Outcome Assessment						

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Study Citation:	E. Nutley, A. Tcheong, J. Allen, B. Collins, M. Ma, X. Lowe, J. Bishop, D. Moore, A. Wyrobek (1996). Micronuclei induced in round spermatids of mice after stem-cell treatment with chloral hydrate: evaluations with centromeric DNA probes and kinetochore antibodies Environmental and Molecular Mutagenesis, 28(2,2), 80-89
Data Type:	Micronuclei in round spermatids of mice
HERO ID:	707248

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Two sensitive methods were used to characterize spermatid micronuclei.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were measured consistently across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	3000 - 6000 cells scored/group
	Metric 19:	Blinding of Assessors	High	× 1	1	Coded slides were scored.
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the negative control group was reported and appeared appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food/water intake were not reported,
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Pair-wise and trend tests were described.
	Metric 24:	Reporting of Data	High	× 2	2	Data were fully reported.
Overall Quality Determination‡			High		1.6	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 205: **Animal toxicity evaluation results for Robbiano et al 2004 for rat renal cell micronucleus study**

Study Citation:	L. Robbiano, D. Baroni, R. Carrozzino, E. Mereto, G. Brambilla (2004). DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney Toxicology, 204(2-3,2-3), 187-195
Data Type:	TCE induced MN in Rat kidneys
HERO ID:	707588

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was reported by name
	Metric 2:	Test Substance Source	High	× 1	1	The test substance source was reported as E. Merk (Darmstadt Germany), batch was not reported
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was reported as reagent grade
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative controls were implied (results were ratio of treated to control) however it is unclear if it is untreated or vehicle.
	Metric 5:	Positive Controls	High	× 1	1	Positive control NDMA was reported and appropriate.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation was not specified; method was previously cited
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Methods were previously cited and briefly described
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	Methods were previously cited and briefly described
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose was clearly reported in mg/kg
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single administration, sacrificed after 2 days was appropriate for the study type
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Number of exposure groups was one and was determined by 1/2 LD50
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route and method was reported (oral) and was suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex and starting body weight were reported and the test animal was obtained from a commercial source. Health status and age were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle) and were adequate .
	Metric 15:	Number per Group	Medium	× 1	2	Number of animals per group was reported as 3 and is less than recommended for the study type but is sufficient for the outcome analysis.

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Study Citation:	L. Robbiano, D. Baroni, R. Carrozzino, E. Mereto, G. Brambilla (2004). DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney Toxicology, 204(2-3,2-3), 187-195
Data Type:	TCE induced MN in Rat kidneys
HERO ID:	707588

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Outcome assessment methodology was cited to a previous publication (Robbiano et al. 1997).
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Outcome assessment methodology was cited to a previous publication (Robbiano et al. 1997).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Sampling adequacy was previously cited and not reported in the brief description.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	not applicable to the study type
	Metric 20:	Negative Control Response	High	× 1	1	negative control response (as reported in figure 1 legend) appeared adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	confounding variables in test design and procedures was not reported
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	confounding variables in outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was conducted, but was unclear. Data presented was sufficient for an independent analysis.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all treated groups (in figure 1) and controls in figure 1 legend.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 206: **Animal toxicity evaluation results for Hrelia et al 1994 for mouse micronucleus study**

Study Citation:	P. Hrelia, F. Maffei, F. Vigagni, C. Fimognari, P. Flori, R. Stanzani, G. C. Forti (1994). Interactive effects between trichloroethylene and pesticides at metabolic and genetic level in mice Environmental Health Perspectives, 102(Suppl 9,Suppl 9), 31-34
Data Type:	In vivo micronucleus assay in CD1 mice
HERO ID:	63884

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as trichloroethylene (TRI)
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified. The product number and batch/lot number were not reported; however, the material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls and solvent (corn oil) controls were tested.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control group was not included in this study; however, it may not be strictly necessary for this in vivo study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation methodology was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation was reported; the solutions were made immediately before administration and did not need to be stored.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentration were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for this endpoint. Single i.p. dose of TRI-alone treated mice compared against controls.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Though the study authors did not justify the number of exposure groups or concentration, the single i.p. exposure level appears to be adequate to show results relevant to the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route was appropriate for the test substance.
Domain 4: Test Organism						

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Study Citation:	P. Hrelia, F. Maffei, F. Vigagni, C. Fimognari, P. Flori, R. Stanzani, G. C. Forti (1994). Interactive effects between trichloroethylene and pesticides at metabolic and genetic level in mice Environmental Health Perspectives, 102(Suppl 9,Suppl 9), 31-34
Data Type:	In vivo micronucleus assay in CD1 mice
HERO ID:	63884

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain, sex, age, and starting body weight were reported. The test animal was from a reported commercial source. The test species and strain were an appropriate animal model for the evaluation of this endpoint.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions were reported and were similar for all groups; The light-dark cycle was specified. It was noted that the animals were housed in facilities with temperature and humidity controls, though the values were not specifically reported. These limitations are unlikely to have a substantial impact on results.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported, appropriate for the study type and outcome analysis (5/group).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest (1000 polychromatic erythrocytes per animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for this study
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the negative control groups were adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food and water consumption were not reported, for each group.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for the dataset.
	Metric 24:	Reporting of Data	High	× 2	2	All data were adequately reported.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	P. Hrelia, F. Maffei, F. Vigagni, C. Fimognari, P. Flori, R. Stanzani, G. C. Forti (1994). Interactive effects between trichloroethylene and pesticides at metabolic and genetic level in mice Environmental Health Perspectives, 102(Suppl 9,Suppl 9), 31-34
Data Type:	In vivo micronucleus assay in CD1 mice
HERO ID:	63884

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 207: **Animal toxicity evaluation results for Robbiano et al 2004 for rat renal cell DNA fragmentation study**

Study Citation:	L. Robbiano, D. Baroni, R. Carrozzino, E. Mereto, G. Brambilla (2004). DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney Toxicology, 204(2-3,2-3), 187-195
Data Type:	DNA fragmentation in Rat kidneys
HERO ID:	707588

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was reported by name
	Metric 2:	Test Substance Source	High	× 1	1	The test substance source was reported as E. Merk (Darmstadt Germany), batch was not reported
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was reported as reagent grade
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Negative controls were implied (results were ratio of treated to control); however, it is unclear if it is untreated or vehicle.
	Metric 5:	Positive Controls	High	× 1	1	Positive control NDMA was reported and appropriate
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation was not specified as method was previously cited
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Methods were previously cited and briefly described
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	Methods were previously cited and briefly described
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	dose was clearly reported in mg/kg
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	single administration, sacrificed after 2 days was appropriate for the study type
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	number of exposure groups was one and was determined by 1/2 LD50
	Metric 12:	Exposure Route and Method	High	× 1	1	the exposure route and method was reported (oral) and was suited to the test substance
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex and starting body weight were reported and the test animal was obtained from a commercial source. Health status and age were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle) and were adequate.
	Metric 15:	Number per Group	Medium	× 1	2	Number of animals per group was reported as 3 and is less than recommended for the study type but is sufficient for the outcome .

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Study Citation:	L. Robbiano, D. Baroni, R. Carrozzino, E. Mereto, G. Brambilla (2004). DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney Toxicology, 204(2-3,2-3), 187-195
Data Type:	DNA fragmentation in Rat kidneys
HERO ID:	707588

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Outcome assessment methodology was cited to a previous publication (Robbiano et al. 1997).
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Outcome assessment methodology was cited to a previous publication (Robbiano et al. 1997).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	not applicable to the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	not applicable to the study type
	Metric 20:	Negative Control Response	High	× 1	1	negative control response (as reported in figure 1 legend) appeared adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	confounding variables in test design and procedures was not reported
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	confounding variables in outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was conducted, but was unclear. Data presented was sufficient for an independent analysis.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all treated groups (in figure 1) and controls in figure 1 legend.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 208: **Animal toxicity evaluation results for Robbiano et al 1998 for single-dose rat study on micronuclei**

Study Citation:	Robbiano, L., Mereto, E., Migliazzi Morando, A., Pastore, P., Brambilla, G. (1998). Increased frequency of micronucleated kidney cells in rats exposed to halogenated anaesthetics Mutation Research, 413(1,1), 1-6
Data Type:	Micronuclei
HERO ID:	707589

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as trichloroethylene (TCE); CASRN was provided.
	Metric 2:	Test Substance Source	Low	× 1	3	Commercial source was not given.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity 99.5%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative vehicle (corn oil) controls were used.
	Metric 5:	Positive Controls	High	× 1	1	Positive (NDMA) control was used.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	TCE was dissolved in corn oil before administration. Storage conditions werenot described.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	All groups received 0.01ml/g
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The dose was reported in mmol/kg.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Animals were given a single oral dose and sacrificed after 48 hrs.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Use of a single dose can be justified in some cases. The dose was reported to be 1/7 of the oral LD50, however no other justification was provided.
	Metric 12:	Exposure Route and Method	High	× 1	1	Oral gavage in corn oil
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species, strain, sex, and starting body weights were reported. - The source, health status and age were not included.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were not reported.
	Metric 15:	Number per Group	High	× 1	1	Seven animals in the treatment group (16/ neg control; 4/positive control)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment was sufficiently described and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent across groups

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Study Citation:	Robbiano, L., Mereto, E., Migliazzi Morando, A., Pastore, P., Brambilla, G. (1998). Increased frequency of micronucleated kidney cells in rats exposed to halogenated anaesthetics Mutation Research, 413(1,1), 1-6
Data Type:	Micronuclei
HERO ID:	707589

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	High	× 1	1	Minimum of 2000 cells/rat
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not reported.
	Metric 20:	Negative Control Response	High	× 1	1	The negative control response was reported and as expected.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Confounding variables related to test design and procedures were not reported
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical significance was assessed using a Wilcoxon two-sample test
	Metric 24:	Reporting of Data	High	× 2	2	Individual animal data, as well as overall means and SD was reported.
Overall Quality Determination‡			High		1.6	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 209: **Animal toxicity evaluation results for Russo and Levis 1992 for aneuploidy in male mouse germ cells**

Study Citation:	A. Russo, A. G. Levis (1992). Detection of aneuploidy in male germ cells of mice by means of a meiotic micronucleus assay Mutation Research, 281(3,3), 187-191
Data Type:	aneuploidy in male mouse germ cells
HERO ID:	707674

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chloral hydrate identified by established nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer (Fluka Chemie AG) was reported. Batch/lot number was not give, but the composition is not expected to vary.
	Metric 3:	Test Substance Purity	High	× 1	1	99% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Study authors acknowledged using a concurrent negative control group, but details regarding the negative control group were not reported.
	Metric 5:	Positive Controls	High	× 1	1	Adriamycin and mitomycin C were concurrent positive controls
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation was described and the test substance was immediately used (no storage).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was consistent across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (mg/kg bw).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single dose; 14-day follow up.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single dose; no justification provided; result was negative so it is unclear if it was sufficiently high.
	Metric 12:	Exposure Route and Method	Low	× 1	3	I.p. injection is acceptable but not a recommended exposure route.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain, sex, and age were reported. Health status and body weight were not given.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	Low	× 1	3	2/group; 5/group recommended
Domain 5: Outcome Assessment						

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Study Citation:	A. Russo, A. G. Levis (1992). Detection of aneuploidy in male germ cells of mice by means of a meiotic micronucleus assay Mutation Research, 281(3,3), 187-191
Data Type:	aneuploidy in male mouse germ cells
HERO ID:	707674

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The method was briefly reported with reference to a published method.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Outcome assessment was partially cited to a published method.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	800-1000 spermatids/animal were scored
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Outcome assessment was briefly reported with reference to a published method. No information on blinding/coding of slides was provided.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control response was reported and appeared appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food/water intake were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical test (G test for MN frequencies) was reported and appeared appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for micronuclei induction (mean and SE and n speramatids) and size.
Overall Quality Determination‡			Medium		1.9	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 210: **Animal toxicity evaluation results for Xu and Adler 1990 for mouse bone marrow aneuploidy study**

Study Citation:	W. Xu, I. Adler (1990). Clastogenic effects of known and suspect spindle poisons studied by chromosome analysis in mouse bone marrow cells Mutagenesis, 5(4,4), 371-374
Data Type:	Aneuploidy in mouse bone marrow cells for chloral hydrate
HERO ID:	708493

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name as "chloralhydrate".
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance (Sigma Chemical) was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent solvent controls were utilized.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design. Positive results were obtained with other compounds in the study.
	Metric 6:	Randomized Allocation	Low	× 1	3	Method of allocation of animals to experimental groups was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The test substance preparation was reported and appropriate. Test substance was freshly prepared directly prior to use.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (mg/kg bw).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were appropriate for this endpoint (single-dose; sample collection at 6, 12, 24, and 36 hr post-injection).
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups and dose spacing was reported (2 plus control at 1.5x spacing; 3 plus control and 2x spacing recommended)
	Metric 12:	Exposure Route and Method	Low	× 1	3	The exposure route (i.p. injection) was acceptable but not recommended for the outcome.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The test animal species, strain, sex, age, and starting body weight range were reported. The test animal commercial source was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.

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Study Citation:	W. Xu, I. Adler (1990). Clastogenic effects of known and suspect spindle poisons studied by chromosome analysis in mouse bone marrow cells Mutagenesis, 5(4,4), 371-374
Data Type:	Aneuploidy in mouse bone marrow cells for chloral hydrate
HERO ID:	708493

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	The number of treated animals per group was appropriate for these endpoints (n = 5/sex) but only one control animal per sex per group was used.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodologies were partially reported appropriate for the endpoints of interest. Bone marrow cell preparation was cited to another publication
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Sampling was low for the outcomes of interest (50 well-spread cells at mitotic metaphase; 200 recommended).
	Metric 19:	Blinding of Assessors	Low	× 1	3	Coding of slides prior to examination was not reported
	Metric 20:	Negative Control Response	High	× 1	1	Responses in negative control groups were reported and appeared appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weight range was reported. Food and water consumption were not reported, but this is not expected to significantly impact the results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The data were analyzed appropriately for each endpoint (Chi-square test with Yates' correction when applicable).
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and groups, including mean, SE, and n/group
Overall Quality Determination‡			Medium		1.8	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study.

Table 211: **Animal toxicity evaluation results for Zordan et al 1994 for drosophila wing spot assay study**

Study Citation:	M. Zordan, M. Osti, M. Pesce, R. Costa (1994). Chloral hydrate is recombinogenic in the wing spot test in Drosophila melanogaster Mutation Research, 322(2,2), 111-116
Data Type:	Drosophila wing spot assay for chloral hydrate
HERO ID:	708586

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name as chloral hydrate with the correct CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance (Prolabo) was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The test substance purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (water).
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	This metric is not applicable to the study design (all larvae grown on test substance-treated medium were included for that experimental group).
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported. Test substance storage was not reported (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations (mM) were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration (larval to pupation stages) were reported and appropriate for this endpoint.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups (4 plus control) and dose spacing were appropriate for this study design.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure (incorporation into larval feed) were appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, age, and sex of the test animals were identified. The commercial source of the test animals was not identified, but crosses utilized in the present study were generated by the study authors.

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Study Citation:	M. Zordan, M. Osti, M. Pesce, R. Costa (1994). Chloral hydrate is recombinogenic in the wing spot test in Drosophila melanogaster Mutation Research, 322(2,2), 111-116
Data Type:	Drosophila wing spot assay for chloral hydrate
HERO ID:	708586

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	The husbandry conditions were adequately reported.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was adequate (37-200+ wings depending on treatment group).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for this endpoint.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this study design (wings per fly).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to this study type.
	Metric 20:	Negative Control Response	High	× 1	1	Responses in negative controls were reported and appeared appropriate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Not Rated	NA	NA	This metric is not applicable to this test organism.
	Metric 22:	Health Outcomes Unrelated to Exposure	Not Rated	NA	NA	This metric is not applicable to this test organism.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Data were appropriately analyzed by one-sided Chi-square test.
	Metric 24:	Reporting of Data	High	× 2	2	All data were reported for all exposure groups and experiments; data included spot frequency and size.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 212: **Animal toxicity evaluation results for Russo et al 1992 for micronucleus study**

Study Citation:	A. Russo, A. Stocco, F. Majone (1992). Identification of kinetochore-containing (CREST+) micronuclei in mouse bone marrow erythrocytes Mutagenesis, 7(3,3), 195-197
Data Type:	In vivo CREST/micronucleus assay for chloral hydrate
HERO ID:	724494

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name as chloral hydrate.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source (Fluka Chemie AG) of the test substance was identified.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Untreated animals were included as concurrent negative controls.
	Metric 5:	Positive Controls	High	× 1	1	Animals treated with mitomycin C were included as positive controls. Chloral hydrate was also intended to be a positive control to validate this assay.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation methods were not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported. Test substance storage was not reported, but is unlikely to significantly impact results (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Negative controls were left untreated. Positive controls and treated animals (only one dose level of chloral hydrate) were administered equal injection volumes (0.01 mL/g body weight).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (mg/kg bw)
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure duration was reported and appropriate for this endpoint (single administration)
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only one dose of chloral hydrate plus control were utilized; dose was justified based on prior published data
	Metric 12:	Exposure Route and Method	Low	× 1	3	Exposure route (i.p. injection) is acceptable but not recommended for endpoint
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, and age were reported. Starting body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions were briefly described (except humidity) and were appropriate.

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Study Citation:	A. Russo, A. Stocco, F. Majone (1992). Identification of kinetochore-containing (CREST+) micronuclei in mouse bone marrow erythrocytes Mutagenesis, 7(3,3), 195-197
Data Type:	In vivo CREST/micronucleus assay for chloral hydrate
HERO ID:	724494

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	3 treated and 2 control mice used per chemical (5 recommended)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was described and appropriate for this endpoint.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across control and treated groups.
	Metric 18:	Sampling Adequacy	Low	× 1	3	The sampling per animal (2,000 polychromatic erythrocytes per animal) was lower than recommended (4000).
	Metric 19:	Blinding of Assessors	Low	× 1	3	Use of coded slides was not reported
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses were reported and appeared appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weight and food/water intake were not reported but unlikely to significantly impact results
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Data were analyzed by G-test.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all groups (mean, SE, and n/grp)
Overall Quality Determination‡			Medium		1.7	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

7 Developmental and Reproductive

Table 213: **Animal toxicity evaluation results of Taylor et al 1985 for a developmental toxicity (oral) study on growth (early life) and development, neurological/behavior outcomes**

Study Citation:	Taylor, DH; Lagory, KE; Zaccaro, DJ; Pfohl, RJ; Laurie, RD (1985). Effect of trichloroethylene on the exploratory and locomotor activity of rats exposed during development Science of the Total Environment, 47(0), 415-420
Data Type:	Developmental toxicity (oral)
HERO ID:	65163

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively (by chemical name).
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of TCE was not identified, but I would not expect it to have a substantial impact on results.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and grade were not reported so I downgraded the score of this metric to low.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative control group was used and was appropriate.
	Metric 5:	Positive Controls	Not Rated	NA	NA	N/A - Positive control is not indicated by the study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	The test substance preparation (in drinking water) and storage (in drinking water bottles) were reported. The test substance in the drinking water bottles was changed every three days and was evaluated for degradation in the water bottles using a gas chromatograph to estimate dosages received. The study report did not mention how frequently the test substance was prepared in the vehicle (drinking water) and it is unclear if it was prepared as frequently as the bottles were changed (i.e., every 3 days).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures in the drinking water were consistent among the groups. No differences were reported that would be suggestive of inconsistencies in exposure administration.

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Study Citation:	Taylor, DH; Lagory, KE; Zaccaro, DJ; Pfohl, RJ; Laurie, RD (1985). Effect of trichloroethylene on the exploratory and locomotor activity of rats exposed during development Science of the Total Environment, 47(0), 415-420
Data Type:	Developmental toxicity (oral)
HERO ID:	65163

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Total consumption of TCE from drinking water was reported (in mg) and was calculated from mean water consumption values and the mean concentrations in drinking water (measured by gas chromatography); however, actual drinking water consumption values were not reported so independent calculations are not possible. Point estimates for consumed TCE in both mg/L and mg were provided. The range of drinking water and TCE degradation data that were the basis of these values were not provided., but the relevant values were reported in the end. Dose information or body weight values used to calculate dosage were not provided, this had to be independently estimated by EPA and cannot be confidently calculated due to an absence of dam vs pup-specific body weight and ingestion values.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were reported and appropriate for this study type and outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups was acceptable; however, deficiencies were apparent in the selection of concentrations, based on the observation of adverse effects at all concentrations (for example, Fig 2a, significantly increased exploratory events at all concentrations), suggesting that the lowest concentration was not low enough. Additionally, the dose/concentration spacing may have been too small (see Fig 2a, responses were quantitatively similar). The lowest dose group was not low enough and a dose-responsive increase was not consistently observed, however the number of groups and spacing were sufficient to show results relevant results.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The route and method of exposure (in drinking water) were reported and were appropriate. Due to the volatility of TCE, drinking water studies ideally should include more controls and more rapid water changes.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The test animal species, strain, and sex were reported; however, life-stage, source, and starting body weight were not reported, so I downgraded the score to low. Age of the offspring was reported for the experimental assays, but age was not reported for dams.

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Study Citation: Taylor, DH; Lagory, KE; Zaccaro, DJ; Pfohl, RJ; Laurie, RD (1985). Effect of trichloroethylene on the exploratory and locomotor activity of rats exposed during development Science of the Total Environment, 47(0), 415-420
Data Type: Developmental toxicity (oral)
HERO ID: 65163

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and similar among the groups. The authors discuss analyzing the effect of time and day effects on outcomes and the fact that motor activity was only assessed at night, and water was provided ad libitum to reduce the effect of differences in dosing timing..
	Metric 15:	Number per Group	Low	× 1	3	The number of maternal animals treated per group (no outcomes reported for maternal animals) was not reported. For pups, the study authors stated that the litters were culled to 8 pups/litter, and the culled male pups were used as the subjects but the number used for each outcome evaluated was not specifically stated; however, a sufficient number was available for statistical analysis, so I considered this metric acceptable, but scored it as low under the assumption that 8 pups were evaluated from each litter.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology only partially addressed the intended outcomes of interest. For pups, while the study evaluated neurological outcomes, specifically, behavior, no post-mortem analyses of pups were performed to evaluate whether there were any abnormalities in neurological tissues (e.g., brain, spinal cord).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	There were some reporting deficiencies in the timing of outcome assessments across study groups, however the authors mention that all studies were carried out at night and they statistically account for effects of any differences in timing.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding sampling of outcomes were not reported and it is not clear that pup data were evaluated on a per litter basis. For example, sampling based on litters was not reported so it is unclear if the means were for all animals evaluated (8 male pups/litter, by litter, i.e., means for litters, or for individual pups).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	The study authors did not report whether assessors were blinded to treatment group for subjective outcomes (e.g., exploratory behavior). All measurements were made electronically, so no blinding was needed.

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Study Citation:	Taylor, DH; Lagory, KE; Zaccaro, DJ; Pfohl, RJ; Laurie, RD (1985). Effect of trichloroethylene on the exploratory and locomotor activity of rats exposed during development Science of the Total Environment, 47(0), 415-420
Data Type:	Developmental toxicity (oral)
HERO ID:	65163

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food/water intake, and respiratory rate were not reported
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and health outcomes unrelated to exposure for each study group were not reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were partially described; the authors discuss using multi-factor ANOVA. Statistical significance is reported for all metrics.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data were reported with some limitations apparent in the graphs for the outcomes of interest. A proper legend is also not provided for the graphs, so coding of data is unclear.
Overall Quality Determination‡			~~Medium~~ ⟶ Low§		~~2.1~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "The study was downgraded due to the following: 1) Actual doses were not reported, and cannot be calculated due to lack of body weight data and lack of dam vs pup water intake data (metric 9); 2) Concerns over sampling (lack of litter distribution data within groups); 3) Use of pup as statistical unit; and 4)Lack of general health assessment in parental animals and neonates"

Table 214: **Animal toxicity evaluation results of Kumar et al 2000 for a short-term and subchronic inhalation study on reproductive outcomes**

Study Citation:	Kumar, P; Prasad, AK; Saxena, DK; Manu, U; Maji, BK; Dutta, KK (2000). Fertility and general reproduction studies in trichloroethylene exposed rats Indian Journal of Occupational Health, 43(3), 117-126
Data Type:	short-term and subchronic inhalation studies (repro)
HERO ID:	724893

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name
	Metric 2:	Test Substance Source	Medium	× 1	2	Commercial source was identified , but not lot. no.
	Metric 3:	Test Substance Purity	High	× 1	1	99% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Compressed air controls for each exposure duration
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not used for this study type
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Exposure details were reported in another publication.
	Metric 8:	Consistency of Exposure Administration	Not Rated	NA	NA	Exposure details were reported in another publication.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Measured concentration was provided without variance. Target concentration was not given. Measurement method was reported in a different paper.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	4hr/day instead of 6 hr/day.. The exposure duration and follow up time varied; however, a 10 week exposure without follow up time was included.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only one concentration level was used.
	Metric 12:	Exposure Route and Method	Not Rated	NA	NA	Exposure details were reported in another paper (air exchanges, condensation).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain, sex, age, and lifestage were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	CK: Well described
	Metric 15:	Number per Group	Medium	× 1	2	6/group is low for assessment of reproductive outcomes.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	

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Study Citation:	Kumar, P; Prasad, AK; Saxena, DK; Manu, U; Maji, BK; Dutta, KK (2000). Fertility and general reproduction studies in trichloroethylene exposed rats Indian Journal of Occupational Health, 43(3), 117-126
Data Type:	short-term and subchronic inhalation studies (repro)
HERO ID:	724893

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	Methods suggest that all outcomes were measured in every animal.
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported, but outcomes were not subjective.
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not reported. TCE is anticipated to be a respiratory irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No deaths or infections were reported..
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	CK: Statistical data were analyzed by students 't' test as modified by Fischer and $p<0.05$ was considered to be significant.
	Metric 24:	Reporting of Data	Low	× 2	6	The incidence of testes lesion was not reported. Table 1 provides the mean, but not the SE as indicated.
Overall Quality Determination‡			Medium		1.7	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 215: **Animal toxicity evaluation results of Duteaux et al 2004 for a 14 day drinking water study in rats on reproductive outcomes**

Study Citation:	Duteaux, S.B., Berger, T., Hess, R.A., Sartini, B.L., Miller, M.G. (2004). Male reproductive toxicity of trichloroethylene: Sperm protein oxidation and decreased fertilizing ability Biology of Reproduction, 70(5), 1518-1526
Data Type:	14 d drinking water study in rats
HERO ID:	733498

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance source identified but without identity certification or analytical verification.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance described as analytical grade. CK: Batch/lot number not provided
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative control receiving vehicle (water) was reported. TCE was administered as a solution of 3% ethoxylated castor oil and controls were not exposed to this vehicle; however, this is not expected to markedly alter the results.
	Metric 5:	Positive Controls	High	× 1	1	A positive control was used to validate finding of oxidized sperm proteins.
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Limited detail on preparation methods were reported and storage methods were not reported; in addition, stability of the test substance in the drinking water solution was not evaluated.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Water intake by exposure group was not reported, but an average water intake (presumably across groups) was reported.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Authors reported doses estimated based on an average water intake across groups, that may not account for intake differences across groups. Doses in the two groups were reported as ranges. Water concentrations were not verified analytically.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Frequency was not reported but assumed to be 7 d/wk because administration was by drinking water. Duration was adequate to observe an effect.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 nonzero dose groups were used; it is unclear if the lowest dose was low enough but number and spacing was adequate to show dose-response relationship.

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Study Citation: Duteaux, S.B., Berger, T., Hess, R.A., Sartini, B.L., Miller, M.G. (2004). Male reproductive toxicity of trichloroethylene: Sperm protein oxidation and decreased fertilizing ability Biology of Reproduction, 70(5), 1518-1526
Data Type: 14 d drinking water study in rats
HERO ID: 733498

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	There are minor limitations to the use of drinking water administration of TCE (volatilization, degradation) but researchers took steps to minimize impact (water prepared fresh daily to minimize headspace in water bottles and limit degradation.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Test animal source, species, and strain were reported; however, ages of the male rats were not reported and could impact sperm parameters.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most animal husbandry conditions were reported and adequate, but animal housing (cage type and number per cage) was not reported.
	Metric 15:	Number per Group	Low	× 1	3	Number per group was not reported for some endpoints, but for most endpoints only 3 rats per group were evaluated.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment was reported in detail and included relatively sensitive endpoints (histology of testes and epididymides, sperm parameters, oxidized proteins on spermatozoa
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Authors noted possibility that IVF technicians of different proficiencies may have influenced those results, because there was substantial variability in control results between trials. No other inconsistencies in outcome assessment were noted.
	Metric 18:	Sampling Adequacy	High	× 1	1	Outcome assessment sample sizes were consistent across exposure groups.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Most endpoints were not subjective, and blinding is not typical for initial histopathology review.
	Metric 20:	Negative Control Response	Medium	× 1	2	Authors noted substantial variability in control groups between IVF trials.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weights differed across exposure groups; high dose rats weighed ~10% more than controls. In addition, the exposed groups gained far less weight (18-19 g vs 78 g in controls), possibly due to the higher starting weight. It is possible that the differences in starting body weights and g reflected age differences (starting age of males not reported), which could influence outcomes.

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Study Citation:	Duteaux, S.B., Berger, T., Hess, R.A., Sartini, B.L., Miller, M.G. (2004). Male reproductive toxicity of trichloroethylene: Sperm protein oxidation and decreased fertilizing ability Biology of Reproduction, 70(5), 1518-1526
Data Type:	14 d drinking water study in rats
HERO ID:	733498

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	No information on attrition, and little information on health outcomes other than measured endpoints was reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was performed, described in detail, and appeared to be appropriate for the endpoints.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data on most endpoints were presented quantitatively (often graphically) and with measures of variability where appropriate. Incidences of histopathology findings were not reported.
Overall Quality Determination‡			~~Medium~~ ⟶ Low§		~~2.1~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Group sizes are very small (n=3 for most endpoints), starting age was not reported and could affect outcomes, and starting BWs differed across groups."

Table 216: **Animal toxicity evaluation results of Blossom et al 2013 for a neurodevelopmental drinking water study in mice on neurological/behavior outcomes**

Study Citation:	Blossom, S. J., Cooney, C. A., Melnyk, S. B., Rau, J. L., Swearingen, C. J., Wessinger, W. D. (2013). Metabolic changes and DNA hypomethylation in cerebellum are associated with behavioral alterations in mice exposed to trichloroethylene postnatally Toxicology and Applied Pharmacology, 269(3), 263-269
Data Type:	Neurodevelopmental drinking water study
HERO ID:	1646059

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name.
	Metric 2:	Test Substance Source	Medium	× 1	2	Manufacturer was identified without lot. number. No analytical verification.
	Metric 3:	Test Substance Purity	High	× 1	1	99+% purity
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Vehicle controls were used (drinking water with castor oil emulsifier).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not used in neurotoxicity tests.
	Metric 6:	Randomized Allocation	High	× 1	1	Dams were randomly assigned; pups were randomly selected.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Test substance preparation was described (TCE was suspended in drinking water with emulsifier (made fresh every 2-3 day). Storage of TCE was not described.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were calculated by study authors based on drinking water consumption.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Continuous exposure to TCE in drinking water PND0 to PND42 (early postnatal period)
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Concentration justified by previous publications.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	It is unclear how much TCE would be lost to volatilization or degradation. This potential problem was partially mitigated by providing freshly solutions every 2 to 3 days.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	MRL +/+ mice were genetically modified and were considered sensitive to TCE neurotoxicity. CK; Authors justified to use genetically modified mice for the current study with a published research

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Study Citation:	Blossom, S. J., Cooney, C. A., Melnyk, S. B., Rau, J. L., Swearingen, C. J., Wessinger, W. D. (2013). Metabolic changes and DNA hypomethylation in cerebellum are associated with behavioral alterations in mice exposed to trichloroethylene postnatally Toxicology and Applied Pharmacology, 269(3), 263-269
Data Type:	Neurodevelopmental drinking water study
HERO ID:	1646059

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	High	× 1	1	8-9 dams per group; 1 male mouse from each litter.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Mechanisitic data (oxidative stress, DNA methylation) and neurobehavior. No histopathology or other neurological measures..
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding sampling were not reported for all outcomes.
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported, but outcomes were not subjective.
	Metric 20:	Negative Control Response	Low	× 1	3	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Body weight and water consumption were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	
	Metric 24:	Reporting of Data	High	× 2	2	
Overall Quality Determination‡			Medium		1.7	
Extracted			No			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 217: **Animal toxicity evaluation results of Blossom et al 2012 for a postnatal exposure study on neurotoxicity gene expression/omics outcomes**

Study Citation:	Blossom, S.J., Melnyk, S., Cooney, C.A., Gilbert, K.M., James, S.J. (2012). Postnatal exposure to trichloroethylene alters glutathione redox homeostasis, methylation potential, and neurotrophin expression in the mouse hippocampus NeuroToxicology, 33(6), 1518-1527
Data Type:	
HERO ID:	2127871

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substance was clearly identified (by CASRN). CAS RN could not be found in the document, however there is not concern that anything other than pure TCE was used since it was obtained from a manufacturer. Therefore it is not expected to impact results.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (manufacturer).
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was 99+% pure. Any observed effects were highly likely to be due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	An appropriate control group was used. Treatment groups were administered TCE suspended in drinking water with emulsifier; control animals were given water with emulsifier only.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control group was not indicated by study type.
	Metric 6:	Randomized Allocation	High	× 1	1	Dams were randomly assigned to treatment groups; one male was randomly selected from each litter. One male was selected only for serum isolation. 6 full litters of males were used for all other assays.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Omission of storage conditions/stability are unlikely to have a substantial impact on the results. Dams received a freshly made solution of TCE in their drinking water every 2-3 days. TCE was suspended in drinking water with 1% emulsifier (Alkamuls EL-620). No information was provided on stock concentration and measured TCE concentrations were not reported. Adding fresh TCE solution every "2-3 days" may result in inconsistencies, even if all groups are treated similarly.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Drinking water was provided ad libitum.

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Study Citation:	Blossom, S.J., Melnyk, S., Cooney, C.A., Gilbert, K.M., James, S.J. (2012). Postnatal exposure to trichloroethylene alters glutathione redox homeostasis, methylation potential, and neurotrophin expression in the mouse hippocampus NeuroToxicology, 33(6), 1518-1527
Data Type:	
HERO ID:	2127871

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Water consumption (of dams and offspring) and body weights (of offspring) were provided in the primary report; doses in mg/kg-day were reported for PND21 until sacrifice on PND42. TCE levels in dams were not measured from PND1- PND20 out of concern that handling the animals may impact the results. TCE-treated rats exhibited normal growth compared to controls.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported. The exposure period (while not the same as that used in traditional developmental neurotoxicity assays) is appropriate for the outcomes of interest (based on previous studies and the timing of brain development.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The exposure concentrations were based on previous studies of low-level TCE exposures from day 0 through the juvenile period. The doses utilized were adequate to address the purpose of the study (i.e. a range of responses was observed).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	There were presumably limitations regarding the route/method of exposure, but measures were taken by the researchers to mitigate the problem (i.e. the use of an emulsifier). This limitation is unlikely to have a substantial impact on the study results.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The species, strain, sex, and age of the test animals were reported. Minor uncertainties (with respect to starting body weights, health status) in the reporting of test animal characteristics are unlikely to substantially impact the results. The test animal was obtained from a commercial laboratory; the researchers justified why this strain/sex was an appropriate model for the evaluation of the outcomes of interest. Using an especially susceptible strain was well explained and makes sense for analyzing sensitive mechanistic endpoints, however it adds potential caveats to extrapolating results to wild-type strains and in turn, humans.

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Study Citation: Blossom, S.J., Melnyk, S., Cooney, C.A., Gilbert, K.M., James, S.J. (2012). Postnatal exposure to trichloroethylene alters glutathione redox homeostasis, methylation potential, and neurotrophin expression in the mouse hippocampus NeuroToxicology, 33(6), 1518-1527

Data Type:

HERO ID: 2127871

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions (temperature, humidity, light/dark cycles) were not sufficiently reported. It was indicated that pregnant mice were housed in separate cages (offspring conditions not reported). There is no indication that the lack of husbandry data impacted the study results.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals/group (6) is lower than that used for standard developmental neurotoxicity studies; however, data were amenable to statistical analysis, and this limitation is unlikely to have a substantial impact on the results. The low number of litters (not animals) did not impact the study's observation of dose-dependent molecular responses, however it may have masked potential effects on body weight, which did not demonstrate any statistically significant difference.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcomes of interest. The timing of the assessment appeared to be sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was conducted consistently across study groups (e.g. the same time after initial exposure).
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding sampling for the endpoints of interest were reported. Mechanistic data were obtained from one randomly selected male from each litter per treatment group. 6 litters per dose group is very small for statistical analysis, although results were observed in the mechanistic studies. However, nothing was reported concerning technical replicates of each experiment, which is primarily an issue for the biochemical and genetic assays.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were assessed (generally automated measurements).
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control						

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Study Citation:	Blossom, S.J., Melnyk, S., Cooney, C.A., Gilbert, K.M., James, S.J. (2012). Postnatal exposure to trichloroethylene alters glutathione redox homeostasis, methylation potential, and neurotrophin expression in the mouse hippocampus NeuroToxicology, 33(6), 1518-1527
Data Type:	
HERO ID:	2127871

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The study reported decreased water consumption in TCE-treated rats on PNDs 36-42 only; since this effect occurred in the absence of effects on growth/body weights, this limitation is not likely to have a substantial impact on the study results. The animals showed no signs of dehydration.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	No data on attrition/health outcomes unrelated to exposure were reported (only substantial differences among groups were noted).
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for the outcomes of interest.
	Metric 24:	Reporting of Data	High	× 2	2	Data were presented by exposure group. Data for some endpoints (i.e. 3-nitrotyrosine levels in plasma and hippocampus, expression of neurotrophic factors) were presented graphically in the primary report.
Overall Quality Determination‡			High		1.6	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 218: **Animal toxicity evaluation results of Dorfmueller et al 1979 for an inhalation teratogenicity study on growth (early life) and developmental outcomes**

Study Citation:	Dorfmueller, MA; Henne, SP; York, RG; Bornschein, RL; Manson, JM (1979). Evaluation of teratogenicity and behavioral toxicity with inhalation exposure of maternal rats to trichloroethylene Toxicology, 14(2,2), 153-166
Data Type:	Inhalation teratogenicity study
HERO ID:	65242

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Trichloroethylene; technical grade (> 99% TCE with 0.2% epichlorohydrin; trade name NEU-TRI ®)
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported to be Dow Chemical Company; batch/lot number was not reported; the omitted details are unlikely to have a substantial impact on results.
	Metric 3:	Test Substance Purity	High	× 1	1	Technical grade TCE with > 99% TCE; any impurities were not identified, but is unlikely to have a substantial impact on results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent control consisted of filtered air; all conditions equal except chemical exposure
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not rated/applicable for this study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The study reported that animals were randomly allocated into study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation for inhalation exposure in chambers was described; storage conditions were not reported; omission of details are unlikely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	consistent chamber designs, animals/chamber was reported; chamber concentrations of TCE vapor were monitored automatically
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	the test substance vapor concentration was measured analytically every 13 minutes by gas chromatograph at a time weighted average of 1800 + - 200 ppm; actual concentrations were not reported; + - 200 ppm range is > 10% of the target concentration of 1800 ppm. Only chamber concentrations were reported.

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Study Citation: Dorfmueller, MA; Henne, SP; York, RG; Bornschein, RL; Manson, JM (1979). Evaluation of teratogenicity and behavioral toxicity with inhalation exposure of maternal rats to trichloroethylene Toxicology, 14(2,2), 153-166
Data Type: Inhalation teratogenicity study
HERO ID: 65242

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and/or outcome(s) of interest; 6 hours/day, 5 days/week for 2 weeks for pre-mating exposures and 6 hours/day, 7 days/week for gestational exposure up to GD 20.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Single concentration tested; a dose-response could not be determined; concentration was justified as a dose that did not produce level of exposure to be subnarcotic, and to result in the induction of minor histopathologic lesions in adult male rats with a 4-week exposure.. Previous studies in the same laboratory indicated that "exposure to 1800 ppm of TCE for 3 weeks did not significantly alter fertility or mating success". The study was designed to compare effects of exposure at different time points (from pre-mating through gestation); therefore a single concentration may be appropriate.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Downgraded from High to Low: A dynamic whole-body chambers for TCE vapor appears to have been used, however while air flow rate is reported the number of changes per hour are not reported. and there are incomplete details provided on the exposure chamber.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Long-Evans hooded rats were purchased from Charles River Breeding Laboratories; starting body weight was reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most husbandry conditions were reported and were adequate and similar for all groups; humidity was not reported
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported and appropriate for the study type and outcome analysis; 30 rats/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Downgraded to medium: Only external anomalies were examined along with fetal livers for assessment of teratogenicity.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	outcomes appear to be assessed consistently across study groups

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Study Citation: Dorfmueller, MA; Henne, SP; York, RG; Bornschein, RL; Manson, JM (1979). Evaluation of teratogenicity and behavioral toxicity with inhalation exposure of maternal rats to trichloroethylene Toxicology, 14(2,2), 153-166
Data Type: Inhalation teratogenicity study
HERO ID: 65242

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported and the study used adequate sampling for the outcomes of interest
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported; however, lack of blinding is not expected to have a substantial impact on results; activity measurements were measured by automatic methods.
	Metric 20:	Negative Control Response	Low	× 1	3	Downgraded to low: The biological responses of the negative control group was reported. Total incidences of skeletal and soft tissue anomalies reported in the control group pups that were higher than some of the treated groups. differences in outcome between untreated and solvent controls).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among the study groups in initial body weight, food or water intake, or respiratory rate that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported; unlikely to have a substantial impact on results
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for dataset
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group
Overall Quality Determination‡			~~High~~ ⟶ Low§		~~1.5~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study
§ Evaluator's explanation for rating change: "Outcomes did not appear to be exposure duration-responsive, and control responses were sometimes greater than treated groups. When combined with only a single dose being used, it was very difficult to interpret the conclusions of the data."

Table 219: **Animal toxicity evaluation results of Gilbert et al 2014 for a developmental immunotoxicity study in mice on gene expression/omics, hematological and immune outcomes**

Study Citation:	Gilbert, KM; Woodruff, W; Blossom, SJ (2014). Differential immunotoxicity induced by two different windows of developmental trichloroethylene exposure Autoimmune Diseases, 2014 982073
Data Type:	Developmental immunotoxicity (oral-immunotoxicity endpoints)
HERO ID:	2799580

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was not reported and this may have a substantial impact on the results. A previous publication is cited regarding utilization of methods of TCE developmental exposure (ref 30) but it was not stated whether the TCE that was used in this study was the same as used in ref 30, so I downgraded the score to low.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and grade were not reported. A previous publication is cited regarding utilization of methods of TCE developmental exposure (ref 30) but it was not stated whether the TCE that was used in this study was the same as used in ref 30, so I downgraded the score to low.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative control group (drinking water containing chemical used for solubilization of TCE, Alkamulus EL-620) was used and was appropriate.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control is not indicated by the study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The study reported that animals were randomly allocated into study groups (maternal animals were divided into groups using stratified randomization).
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	There were reporting deficiencies in preparation and storage of the test substance. The study authors noted that TCE was solubilized in 1% Alkamuls EL-620 and TCE-containing drinking water (was changed 3 times per week), but no further details were provided on how frequent the test substance was prepared and whether the prepared dosing solutions were stored for any duration of time. Due to these reporting deficiencies, I downgraded the score to low.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures (supplied in drinking water) were administered consistently across study groups.

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Study Citation:	Gilbert, KM; Woodruff, W; Blossom, SJ (2014). Differential immunotoxicity induced by two different windows of developmental trichloroethylene exposure Autoimmune Diseases, 2014 982073
Data Type:	Developmental immunotoxicity (oral-immunotoxicity endpoints)
HERO ID:	2799580

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Deficiences in reporting of administered doses occurred (e.g., no information on animal body weight or intake were provided and concentrations were not measured analytically), but there was no evidence of palatability differences. Based on drinking water intakes, the mean doses of TCE were calculated and reported in mg/kg/day in Figure 1. Due to the uncertainties of reported doses/concentrations, I scored this metric as acceptable, but low.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and the outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and spacing were reported and considered adequate for the purpose of the study. Selected concentrations were justified by the study authors.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and these were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	There were minor uncertainties in reporting of test animal characteristics. The test animal species, strain, sex, age, and source were reported; however, starting body weight and health status were not reported. The MRL+/+ mouse strain, an autoimmune prone strain that is uniquely susceptible to the outcomes of interest reviewed in this form (immune-related assays), was used.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions, including temperature, humidity, light-dark cycle, and housing were not reported; therefore, husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and/or if differences occurred among the groups. These reporting deficiencies are likely to have a substantial impact on results.
	Metric 15:	Number per Group	Medium	× 1	2	The reported number of animals per study group was lower than the typical number used in studies of the same or similar type (in this study, assays were conducted using n = 5-7 litters/treatment group) but sufficient for statistical analysis.
Domain 5: Outcome Assessment						

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Study Citation:	Gilbert, KM; Woodruff, W; Blossom, SJ (2014). Differential immunotoxicity induced by two different windows of developmental trichloroethylene exposure Autoimmune Diseases, 2014 982073
Data Type:	Developmental immunotoxicity (oral-immunotoxicity endpoints)
HERO ID:	2799580

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcomes of interest and was also sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for outcomes of interest were reported and the study used adequate sampling for the outcomes of interest. In this study, the experimental unit was the litter.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not reported and is not considered applicable to the outcomes evaluated.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables were reported; however, initial body weight and food/water intake were not reported. This may have a substantial impact on results since the test substance was administered in drinking water.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted by the study authors.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	The statistical analyses were conducted using the Student's t-test, which may not be appropriate for these data sets (potential for Type 1 error). Sufficient data were provided for data sets that were considered exposure-related to allow an independent reanalysis, if deemed important; however, data were not provided for all of the endpoints evaluated (i.e., those not found to be exposure-related based on the author's statistical methods); therefore, I downgraded the scoring of this metric to low.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group. Negative findings were reported qualitatively and quantitatively for some assays/endpoints.
Overall Quality Determination‡			Medium		1.8	

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Study Citation:	Gilbert, KM; Woodruff, W; Blossom, SJ (2014). Differential immunotoxicity induced by two different windows of developmental trichloroethylene exposure Autoimmune Diseases, 2014 982073
Data Type:	Developmental immunotoxicity (oral-immunotoxicity endpoints)
HERO ID:	2799580

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i \left(\text{Metric Score}_i \times \text{MWF}_i\right) / \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 220: **Animal toxicity evaluation results of Healy et al 1982 for a rat inhalation developmental toxicity study on growth (early life) and development outcomes**

Study Citation:	Healy, TEJ; Poole, TR; Hopper, A (1982). Rat fetal development and maternal exposure to trichloroethylene 100 ppm British Journal of Anaesthesia, 54(3), 337-341
Data Type:	Rat inhalation developmental toxicity study
HERO ID:	65249

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name.
	Metric 2:	Test Substance Source	High	× 1	1	Test material was distilled from Trilene manufactured by ICI.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity of the test substance was not reported, but study reports distilling the commercial product prior to use.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham-exposed controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were allocated to study groups by random number.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	There was no mention of the method and equipment used to generate the test substance. While TCE storage and preparation of TCE in the inhalation chamber was not reported, TCE concentrations were measured suggesting that TCE was not too degraded and there was a light discussion of the inhalation exposure conditions.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Test and control groups were exposed simultaneously using the same room air source; however, there was an incident in which two exposed rats received a high dose of TCE due to an obstruction in the air supply. The authors indicated that these were the only two rats affected by this incident.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Nominal concentration reported; actual concentrations measured but not reported. Actual concentrations of TCE vapor were not reported, although they were measured.

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Study Citation:	Healy, TEJ; Poole, TR; Hopper, A (1982). Rat fetal development and maternal exposure to trichloroethylene 100 ppm British Journal of Anaesthesia, 54(3), 337-341
Data Type:	Rat inhalation developmental toxicity study
HERO ID:	65249

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Exposure frequency was only 4 hours/day compared with typical 6 hours/day. Exposure period began (GD8) and ended (GD21) later than typical (GD6-15). 4hrs/day vs 6hrs/day is a minor limitation and the different gestation exposure length should not too substantially impact results since exposure was for a longer overall duration than standard studies.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	There was only a single exposure group.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The study reported only that stainless steel exposure chambers were used and an air compressor pump was used for air flow. No other details of the exposure chamber were reported.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The test animal source was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Light-dark cycle was not reported; temperature and humidity conditions were adequate and similar between groups.
	Metric 15:	Number per Group	High	× 1	1	Group sizes were 31 and 32
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Fetal soft tissues, including only the liver, kidney, heart, lung diaphragm, and GI system, were examined grossly.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently including at the same time of day and same day or pregnancy.
	Metric 18:	Sampling Adequacy	High	× 1	1	All animals were examined for all endpoints.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	
	Metric 20:	Negative Control Response	High	× 1	1	Control responses are reasonable
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Respiratory rate was not reported; thus it is unknown whether irritation could have triggered reflex bradypnea. KJ: Food/water intake was also not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Two pregnant exposed rats died due to TCE overdose resulting from air supply obstruction occurring once and restricted to these two animals.
Domain 7: Data Presentation and Analysis						

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Study Citation:	Healy, TEJ; Poole, TR; Hopper, A (1982). Rat fetal development and maternal exposure to trichloroethylene 100 ppm British Journal of Anaesthesia, 54(3), 337-341
Data Type:	Rat inhalation developmental toxicity study
HERO ID:	65249

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical methods were reported, and used the litter as the unit of analysis for most endpoints; it is not clear whether the skeletal anomaly incidences were reported on a litter basis.
	Metric 24:	Reporting of Data	Low	× 2	6	Skeletal abnormality incidence was reported without details of the nature of the abnormalities. Other endpoints were reported appropriately. Organ data was also not reported
Overall Quality Determination‡			Unacceptable⋆⋆		2.0	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 221: **Animal toxicity evaluation results of Blossom et al 2016 for a developmental study in mice on growth (early life) and development, neurological/behavior, hematological and immune outcomes**

Study Citation:	Blossom, SJ; Melnyk, SB; Li, M; Wessinger, WD; Cooney, CA (2016). Inflammatory and oxidative stress-related effects associated with neurotoxicity are maintained after exclusively prenatal trichloroethylene exposure NeuroToxicology,
Data Type:	Developmental Neurotox and Immunotox
HERO ID:	3502024

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively as TCE.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance (manufacturer) was reported, but the batch/lot number were not reported.
	Metric 3:	Test Substance Purity	High	× 1	1	>99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle control was used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed for this study design.
	Metric 6:	Randomized Allocation	Medium	× 1	2	Study authors report "stratified randomization". I assume this means body-weight stratified (non-random component).
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Mixed in drinking water with contained 1% Alkamuls EL- 620, an emulsifier consisting of ethoxylated castor oil. It is unclear if water was mixed once for whole study, or multiple times. Study authors predicted 20% degradation in water bottles.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The authors reported the administered doses/concentrations which was calculated based on average water intake, body weight including accounting for ~20% TCE degradation in the water bottles.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Study authors reported that dams exposed "Gestationally" until day of birth. Although they haven't disclosed explicitly the start of exposure it is assumed that the gestational exposure was from GD 0 - PND 0, as per the previous work cited by the study authors (Blossom et al., 2008; Gilbert et al., 2014)."

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Study Citation:	Blossom, SJ; Melnyk, SB; Li, M; Wessinger, WD; Cooney, CA (2016). Inflammatory and oxidative stress-related effects associated with neurotoxicity are maintained after exclusively prenatal trichloroethylene exposure NeuroToxicology,
Data Type:	Developmental Neurotox and Immunotox
HERO ID:	3502024

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose/concentration spacing were justified by study authors and considered adequate to address the purpose of the study.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The authors administered TCE in drinking water wherein an emulsifier was used to solubilize TCE. However, the authors did not report how frequently they made the emulsion, although they factored in ~20% degradation of the chemicals in water bottles, that was added into the dose calculations.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The authors used pregnant female MRL +/+ mice, but did not report their source, age, health status and starting body weight. These deficiencies are likely to have substantial impact on results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and if differences occurred between control and exposed populations.
	Metric 15:	Number per Group	High	× 1	1	8-10 litters/group; 1 male per litter used for testing
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcome(s) of interest and was sensitive for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of outcome assessment protocol were reported all evaluated in all groups. Open field was automated.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcome(s) of interest were reported and the study used adequate sampling for the outcome(s) of interest (e.g., litter data [8-10 litters; 1 male/litter] provided for developmental studies; endpoints were evaluated in an adequate number of animals in each group).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	All endpoints were quantitative
	Metric 20:	Negative Control Response	Low	× 1	3	Control values reported; however, since this is a susceptible strain it is not clear how values would compare to "normal".
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weight not reported, but no change in terminal body weight. No exposure-related effects in drinking water intake.

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Study Citation:	Blossom, SJ; Melnyk, SB; Li, M; Wessinger, WD; Cooney, CA (2016). Inflammatory and oxidative stress-related effects associated with neurotoxicity are maintained after exclusively prenatal trichloroethylene exposure NeuroToxicology,
Data Type:	Developmental Neurotox and Immunotox
HERO ID:	3502024

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Detailed description of statistical analysis were reported.
	Metric 24:	Reporting of Data	High	× 2	2	Quantitative reporting of all findings with exposure-related findings.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 222: **Animal toxicity evaluation results of Shell Oil Co 1980 for a developmental toxicity study in rats and rabbits on growth (early life) and development, adult body weight, respiratory, hepatic, renal, and reproductive outcomes**

Study Citation:	Shell Oil Company (1980). Initial submission: Teratogenic-mutagenic risk of workplace contaminants: Trichloroethylene, perchloroethylene, & carbon disulfide (final report) with attachments and cover letter
Data Type:	Developmental toxicity (rats, rabbits)
HERO ID:	4215763

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was clearly identified.
	Metric 2:	Test Substance Source	High	× 1	1	The test substance source and batch number was provided. Its identity was verified by analytical means.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity and grade were reported and acceptable.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate concurrent negative control group..
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly assigned to exposure groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Study reports storage of the test substance (in drums), but without information on average temperature, although there was no indication of instability of test substance. Methods and equipment for generating of the test atmospheres were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Methods for exposing the study groups to test chemical or the control air were reported and were generally consistent across the study groups. However, control animals were maintained in a different room than the 500 ppm TCE animals during treatments to prevent exposure to TCE.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Nominal test concentrations were reported and mean concentrations were analytically determined during the exposures based on air sampling.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and the outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Unacceptable	× 1	4	This study tested only one quantitative exposure group, 500 ppm, and a control.

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Study Citation:	Shell Oil Company (1980). Initial submission: Teratogenic-mutagenic risk of workplace contaminants: Trichloroethylene, perchloroethylene, & carbon disulfide (final report) with attachments and cover letter
Data Type:	Developmental toxicity (rats, rabbits)
HERO ID:	4215763

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The exposure route (inhalation) and method were appropriate for the test substance. A dynamic whole-body chamber was used for exposures. Air changes per hour were not reported.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Most test animal characteristics (source, species, strain, sex, life stage, and weight range) were reported; however, and health status at the beginning of the study was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported, including temperature, humidity, and light-dark cycle, and were adequate and no differences were reported for the test substance-exposed and control groups.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was sufficient for the study type and outcomes of interest.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate. The length and window of gestational exposure were consistent with studies of similar type.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment methodology addressed the intended outcomes of interest and was sensitive for the outcomes of interest.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	
	Metric 20:	Negative Control Response	High	× 1	1	The negative control response was appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no confounding differences among the study groups in initial body weight. Respiratory rate was not reported, so I scored this as low since TCE is a potential respiratory irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were described and appropriate for the datasets.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were adequately presented (in text and/or tables).

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Study Citation:	Shell Oil Company (1980). Initial submission: Teratogenic-mutagenic risk of workplace contaminants: Trichloroethylene, perchloroethylene, & carbon disulfide (final report) with attachments and cover letter
Data Type:	Developmental toxicity (rats, rabbits)
HERO ID:	4215763

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		~~Unacceptable~~⋆⋆ $\longrightarrow$ Low§		~~1.4~~	
Extracted		No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i \left(\text{Metric Score}_i \times \text{MWF}_i\right) / \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Even though, study authors used only one dose, this study was reasonably well conducted and would provide supporting evidence for the chemical effects."

Table 223: **Animal toxicity evaluation results of Gilbert et al 2017 for a gestation, lactation, and adulthood oral exposure study in mice on growth (early life) and developmental outcomes**

Study Citation:	Gilbert, KM; Bai, S; Barnette, D; Blossom, SJ (2017). Exposure cessation during adulthood did not prevent immunotoxicity caused by developmental exposure to low-level trichloroethylene in drinking water Toxicological Sciences, 157(2), 429-437
Data Type:	Gestation, lactation, adulthood oral exposure in mice-immunotox
HERO ID:	4215946

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Low	× 2	6	Test substance only identified by name
	Metric 2:	Test Substance Source	Low	× 1	3	The source was not identified.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity was not reported. CK: Could able to find in the referred reference (Blossom, S. J., and Doss, J. C. (2007). So, upgraded to Medium
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were used. CK: Could not find Negative control group reported in the study(?).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required.
	Metric 6:	Randomized Allocation	Medium	× 1	2	A stratified randomization process was used, but not additional details were reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Limited details were reported on preparation and storage. No details were reported regarding prevention of loss during preparation.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Duration of exposure was reported and appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Exposures were based on human occupational and environmental exposure levels.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Drinking water was changed 3 times per week to offset degradation.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The source, species, strain, age, and sex were reported. Health status and starting body weight were not reported.

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Study Citation:	Gilbert, KM; Bai, S; Barnette, D; Blossom, SJ (2017). Exposure cessation during adulthood did not prevent immunotoxicity caused by developmental exposure to low-level trichloroethylene in drinking water Toxicological Sciences, 157(2), 429-437
Data Type:	Gestation, lactation, adulthood oral exposure in mice-immunotox
HERO ID:	4215946

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate. Temperature, humidity, light:dark cycle, housing, and feed were not reported. CK: Could able to find in the referred reference (Blossom, S. J., and Doss, J. C. (2007).
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was sufficient for outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology addressed the intended outcome of interest and were sensitive for immunotoxicity. Assessment of other outcomes including weights and litter data were not described.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were consistently assessed across groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Sampling was adequate for the outcomes of interest described.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric was not applicable.
	Metric 20:	Negative Control Response	Medium	× 1	2	Negative control responses were appropriate. CK: Negative controls were not used in the study(?) But vehicle control responses were appropriate. So, Downgraded to
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight was not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical methods were reported with some omissions.
	Metric 24:	Reporting of Data	High	× 2	2	Data for the outcomes of interest were reported.
Overall Quality Determination‡			Medium		1.8	
Extracted			Yes			

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Study Citation:	Gilbert, KM; Bai, S; Barnette, D; Blossom, SJ (2017). Exposure cessation during adulthood did not prevent immunotoxicity caused by developmental exposure to low-level trichloroethylene in drinking water Toxicological Sciences, 157(2), 429-437
Data Type:	Gestation, lactation, adulthood oral exposure in mice-immunotox
HERO ID:	4215946

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 224: **Animal toxicity evaluation results of Charles River Laboratories 2019 for a prenatal drinking water exposure study (gd 1-21) in rats on growth (early life) and developmental outcomes**

Study Citation:	Charles River Laboratories International Inc. (2019). An oral (drinking water) study of the effects of trichloroethylene (TCE) on fetal heart development in Sprague Dawley rats: Laboratory Project ID 00459506
Data Type:	prenatal drinking water exposure (GD 1-21) developmental study in rats
HERO ID:	5035313

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively by chemical name and CAS number
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported, including manufacturer and batch/lot number; certificate of analysis was included in the report
	Metric 3:	Test Substance Purity	High	× 1	1	purity reported in certificate of analysis; 99.98%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	vehicle control; Reverse osmosis-purified water
	Metric 5:	Positive Controls	Low	× 1	3	all-trans Retinoic acid (CAS No. 302-79-4); a well-known characterized developmental toxicant that has been previously demonstrated to result in heart malformations in this strain of rat. The defects observed in the positive control hearts were limited and did not include all of the defects that one would expect to observe after RA treatment (see Fisher 2001, In J Toxicol for an example), suggesting that the scope of the study was narrow.
	Metric 6:	Randomized Allocation	High	× 1	1	Mated females assigned to groups using WIL Toxicology Data Management System (WTDMS™) computer program that assigns animals based on stratification of Gestation Day 0 body weights
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Records of the receipt, distribution, and storage of test substance, and positive controls were maintained; formulations were prepared daily, in a closed (nitrogen purged) system, under amber light, without sonication, and stored and transported in the same closed system.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups in a scientifically sound manner

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Study Citation:	Charles River Laboratories International Inc. (2019). An oral (drinking water) study of the effects of trichloroethylene (TCE) on fetal heart development in Sprague Dawley rats: Laboratory Project ID 00459506
Data Type:	prenatal drinking water exposure (GD 1-21) developmental study in rats
HERO ID:	5035313

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	administered doses/concentrations, or the information to calculate them, were reported without ambiguity; concentration analysis was conducted and reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and outcome of interest; administered the test substance continuously in drinking water from Gestation Day 1 through euthanasia (scheduled for Gestation Day 21).
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose/concentration spacing were justified by study authors and considered adequate to address the purpose of the study; control, positive control, and 4 dose groups
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The route and method of exposure were reported and were suited to the test substance. the positive control was administered via gavage as that route of exposure has been demonstrated a positive response. Although measures were taken to minimize TCE loss (including nitrogen purging to reduce headspace), measures of 24hr loss show >40% loss for most samples. 24hr loss in the Johnson 2003 study that this was attempting to replicate was only 35% on average.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Sprague-Dawley rats
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle) and were adequate and the same for control and exposed populations, such that the only difference was exposure.
	Metric 15:	Number per Group	High	× 1	1	25 females/group
Domain 5: Outcome Assessment						

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Study Citation:	Charles River Laboratories International Inc. (2019). An oral (drinking water) study of the effects of trichloroethylene (TCE) on fetal heart development in Sprague Dawley rats: Laboratory Project ID 00459506
Data Type:	prenatal drinking water exposure (GD 1-21) developmental study in rats
HERO ID:	5035313

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	This study attempts to replicate the experimental conditions of the Johnson 2003 study, however the data from this study indicates that the scope was overly narrow to only focus on ventricular septal defects. The HSIA study states that the fetal evaluation methods were conducted according to Stuckhardt and Poppe (1984), a heart examination method that includes visualization of the valves (the tricuspid, mitral, aortic, and pulmonary valves); however, the HSIA study did not report valve defects in any TCE group or the RA positive control group even though published reports of TCE and RA had identified valve defects. Moreover, the Stuckhardt and Poppe method does not include examination of the heart for atrial septal defects. Accordingly, the HSIA study did not report any atrial septal defects in either the RA positive control group or the TCE groups. Thus, whereas the Johnson et al. (2003) study reported atrial septal defects and valve defects in TCE-exposed fetuses, the HSIA study failed to report these malformations in either the TCE or RA-treated groups.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported and the study used adequate sampling for the outcomes of interest.
	Metric 19:	Blinding of Assessors	High	× 1	1	macroscopic examinations were performed blind to treatment group; Fetal examinations were conducted without knowledge of treatment group..
	Metric 20:	Negative Control Response	Medium	× 1	2	The biological response of the negative control group was adequate, however the percentage of VSDs in the control (2.4%) exceeded both historical control averages (0.26%/litter) and Johnson 2003 (0.66%). This would not be expected to have a significant effect on results.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among the study groups in initial body weight, food or water intake, or respiratory rate that could influence the outcome assessment.

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Study Citation:	Charles River Laboratories International Inc. (2019). An oral (drinking water) study of the effects of trichloroethylene (TCE) on fetal heart development in Sprague Dawley rats: Laboratory Project ID 00459506
Data Type:	prenatal drinking water exposure (GD 1-21) developmental study in rats
HERO ID:	5035313

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	there were no differences among groups that could influence the outcome assessment
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical methods were clearly described and appropriate for datasets, however terminology was often confusingly described. The HSIA final report describes the statistical analysis of the fetal examination findings on page 25 as "Summation per Group (%) = Sum of Viable Fetuses Affected/Litter (%) / No. Litters/Group". This description of the statistical analysis is unclear and does not use customary wording to describe statistical methods for litter-based proportional data. The equation on page 25 of the HSIA report would be more clearly phrased as "Mean % Affected/Litter Per Group = . . . " rather than "Summation per Group", and the third row of text table 14 would be better labeled "Mean % affected per litter" rather than just "% per litter". Additionally, for the positive control group, the table shows 42.2 rather than the correct value, 41.2. These factors did not substantially affect the interpretation of results but could be improved for clarity and accuracy.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data for exposure-related findings were presented for all outcomes by exposure group Data reporting was inconsistent between text and tables, with some observed types of cardiac defects only reported in the text, or only in individual animal tables but not summary tables. These are in addition to the error mentioned above in metric 23: In Text Table 14, the positive control value shown is 42.2%, instead of the correct value of 41.2%. These reporting issues are not major but are problematic for a GLP-style study.
Overall Quality Determination‡			~~High~~ ⟶ Medium§		~~1.4~~	
Extracted			Yes			

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Study Citation:	Charles River Laboratories International Inc. (2019). An oral (drinking water) study of the effects of trichloroethylene (TCE) on fetal heart development in Sprague Dawley rats: Laboratory Project ID 00459506
Data Type:	prenatal drinking water exposure (GD 1-21) developmental study in rats
HERO ID:	5035313

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "The stated purpose of the current study was "to replicate the findings of Dawson et al. and Johnson et al." While this study appears to be robustly performed, the dissection methodology and positive control data indicate that it was inappropriately limited in scope for accomplishing that task. The study appears to have focused only on ventricular septal defects, while insufficiently identifying expected valve and atrial defects. The study is therefore of relatively high quality as a standalone study, but it is downgraded due to being insufficient for its intended purpose. Additionally, there were some reporting inconsistencies that made following the details of all the defects investigated difficult."

Table 225: **Animal toxicity evaluation results of Schwetz et al 1975 for a developmental toxicity study on growth (early life) and development toxicity outcomes**

Study Citation:	Schwetz, BA; Leong, BKJ; Gehring, PJ (1975). The effect of maternally inhaled trichloroethylene, perchloroethylene, methyl chloroform, and methylene chloride on embryonal and fetal development in mice and rats Toxicology and Applied Pharmacology, 32(1), 84-96
Data Type:	Developmental toxicity
HERO ID:	65271

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance source (The Dow Chemical Company) was reported
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was reported (99.240%) and acceptable.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for this study.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study authors did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The method and equipment used for generating the test substance vapors were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Actual concentrations were not reported; however, concentrations were measured three times per day using IR spectrophotometry and chamber concentrations were continuously monitored with a recording combustion analyzer. Analyzed concentrations were stated to be essentially the same as nominal concentrations, so only nominal concentrations were reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and the outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only one quantitative exposure group, 300 ppm, was tested, and a control. A single dose does not make the study unacceptable, however it is just less useful for dose-response assessment

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Study Citation: Schwetz, BA; Leong, BKJ; Gehring, PJ (1975). The effect of maternally inhaled trichloroethylene, perchloroethylene, methyl chloroform, and methylene chloride on embryonal and fetal development in mice and rats Toxicology and Applied Pharmacology, 32(1), 84-96
Data Type: Developmental toxicity
HERO ID: 65271

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	The route was reported, however, it was not stated whether the exposures were nose- or head-only, or whole body. Air changes per hour were not reported.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Most test animal characteristics (source, species, strain, sex, life stage, and weight range) were reported; however, and health status at the beginning of the study was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	The authors mentioned that animals were "housed in a room controlled for temperature, humidity, and light cycle", but did not provide specifics.
	Metric 15:	Number per Group	High	× 1	1	The number of animals (n=12 for TCE) per study group were reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcomes and was sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups (e.g., measurements performed on the same days during the study).
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No evaluations that were considered subjective were conducted, so this metric is considered to be not applicable.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in test design and procedures were reported; however, respiratory rate was not reported although TCE is a potential irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were described and appropriate for the data sets.

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Study Citation:	Schwetz, BA; Leong, BKJ; Gehring, PJ (1975). The effect of maternally inhaled trichloroethylene, perchloroethylene, methyl chloroform, and methylene chloride on embryonal and fetal development in mice and rats Toxicology and Applied Pharmacology, 32(1), 84-96
Data Type:	Developmental toxicity
HERO ID:	65271

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	Medium	× 2	4	Data for exposure-related findings were not presented for all exposure-related outcomes. Decreased maternal body weight was observed in rats exposed to TCE. This is reported qualitatively in the text under results and in a table; however, no quantitative data were provided.
Overall Quality Determination‡		~~High~~ ⟶ Medium§		~~1.7~~	
Extracted		No			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Based on several concerns including missing details on exposure, husbandry, and confounders, the study is downgraded to a medium."

Table 226: **Animal toxicity evaluation results of Beliles et al 1980 for a gestational exposure inhalation study on growth (early life) and development outcomes**

Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	Gestational exposure inhalation
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name and synonym
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer and lot number given.
	Metric 3:	Test Substance Purity	Medium	× 1	2	91% pure, impurities were not characterized (PCE), 99.9% pure for TCE
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Filtered air controls; control animals exposed in a different room.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not used in developmental studies.
	Metric 6:	Randomized Allocation	High	× 1	1	randomly assigned to groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Method and equipment used to generate the test substance as a vapor were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Chambers at 500ppm showed less than 2.5% variation throughout
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and analytical concentrations were provided. Range of measure concentration did not deviate more than 10%.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure throughout gestation or GD 6-18; 7 hours/day.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only 1 exposure concentration was used (500ppm). .
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Dynamic chamber , whole body, it is assumed that the substance does not condense. Number of air changes not indicated
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain and source were reported; starting age and bw not given.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	well reported
	Metric 15:	Number per Group	High	× 1	1	~20/group

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Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	Gestational exposure inhalation
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	Litter data provided for applicable outcome
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported, but most outcomes were not subjective.
	Metric 20:	Negative Control Response	Low	× 1	3	Visceral and skeletal effects seen in controls
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not measured; the chemical is a respiratory irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	subcutaneous hematomas observed in all groups, including controls
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistics were well described and appropriate
	Metric 24:	Reporting of Data	High	× 2	2	All outcome were reported.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 227: **Animal toxicity evaluation results of Fredriksson et al 1993 for a developmental neurotoxicity study on growth (early life) and development outcomes**

Study Citation:	Fredriksson, A; Danielsson, BRG; Eriksson, P (1993). Altered behaviour in adult mice orally exposed to tri- and tetrachloroethylene as neonates Toxicology Letters, 66(1), 13-19
Data Type:	Developmental Neurotoxicity
HERO ID:	196803

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substance was identified definitively, but CASRN, physical nature, physiochemical properties, and/or structure not reported.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance and/or its analytical verification not reported which are likely to have a substantial impact on the results.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of test substance were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors reported using a concurrent negative control group (20% fat emulsion vehicle only) in which all conditions equal except exposure to test substance.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not required for study design.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance preparation was reported; however, its storage was not reported.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were reported, however, gavage volume only reported for controls.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The exposure doses/concentrations or amounts of test substance were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration was reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups and dose/concentration spacing were justified by study authors and considered adequate to address the purpose of the study.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						

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Study Citation: Fredriksson, A; Danielsson, BRG; Eriksson, P (1993). Altered behaviour in adult mice orally exposed to tri- and tetrachloroethylene as neonates Toxicology Letters, 66(1), 13-19
Data Type: Developmental Neurotoxicity
HERO ID: 196803

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, health status, and age were reported, and the test animal was obtained from a commercial source. However, the starting body weight of the pups was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	All husbandry conditions except humidity, were reported.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per study group was reported, that was appropriate for the study type and outcome analysis (12/group), however larger N would have been ideal based on reproductive/developmental toxicity guidelines.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	All endpoints considered developmental since exposure was only pre-weaning (PND 10-16. The outcome assessment methodology addressed or reported the intended outcome(s) of interest and was sensitive for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Low	× 1	3	It is difficult to discern definitively but based on the methods description and a statistical paper published explaining the methods used (Eriksson 2005, The Toxicologist) it appears that the pup was used as a statistical unit. While this is less important because the mice were not exposed in utero, it still ignores known litter effects, as documented in (Holsen et al, 2008). Additionally, Holson et al 2008 recommends examining both sexes, while this study only examines males.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not necessary (neuro assessment was automated)
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group(s) were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weight was not reported, but authors did not report significant differences in weight or weight gain; authors did not report on food or water intake or respiratory rate. Lack of this information is unlikely to have a significant impact on results.

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Study Citation:	Fredriksson, A; Danielsson, BRG; Eriksson, P (1993). Altered behaviour in adult mice orally exposed to tri- and tetrachloroethylene as neonates Toxicology Letters, 66(1), 13-19
Data Type:	Developmental Neurotoxicity
HERO ID:	196803

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical analysis was described, however, data is presented and analyzed group-wise but not by litter-wise. The specifics of analyzing pups as opposed to litters were not explicitly explained, and failing to account for litter effects could have a large statistical impact on results.
	Metric 24:	Reporting of Data	High	× 2	2	Behavioral data at PND 60 reported graphically; qualitative reporting of PND 17 data (with statistics) for lack of exposure-related findings. Study authors do reference Figure 1 for PND 17 data; however, Figure 1 only contains PND 60 data. Qualitative reporting of clinical signs and body weight (no exposure-related findings).
Overall Quality Determination‡			Medium		1.8	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 228: **Animal toxicity evaluation results of Carney et al 2006 for a gestational exposure study in rats on reproductive, growth (early life) and development, nutrition and metabolic/adult exposure body weight, and mortality outcomes**

Study Citation:	Carney, EW; Thorsrud, BA; Dugard, PH; Zablotny, CL (2006). Developmental toxicity studies in Crl:CD (SD) rats following inhalation exposure to trichloroethylene and perchloroethylene Birth Defects Research, Part B: Developmental and Reproductive Toxicology, 77(5), 405-412
Data Type:	Gestational exposure study -TCE
HERO ID:	630415

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	trichloroethylene (TCE) CAS No. 79-01-6)
	Metric 2:	Test Substance Source	High	× 1	1	Dow Chemical Company, no batch number. Batch number not required for discrete chemical and identity was confirmed by analytical methods.
	Metric 3:	Test Substance Purity	High	× 1	1	99.0 +/- 0.05%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate negative control included
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not required by cited guidelines (OPPTS 870.370 and OECD 414)
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly assigned to four groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Detailed vapor generation method reported for TCE. Storage of TCE was not reported, including how frequently it was freshly prepared/changed to a new bottle. Changed to medium.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Consistent methods across group. The concentrations of TCE were measured twice per hour with a Miran 1A infrared spectrometer.Exposure administration consistent across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and analytical exposure levels were reported.. Standard deviation of analytical exposure levels was also reported
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	GD 6-20, 6 hr/d, 7 d/wk; Both guidelines cited indicate that animals should be dosed until the day prior to C-section and sacrifice, which was reported as GD 21.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	3 exposure and 1 control. These test concentrations were based on the results from the previously discussed developmental toxicity studies. The highest exposure level of 600ppm (equivalent to 3.2 TCE/L) exceeds the limit concentration of 2 mg/L specified in the EPA prenatal developmental toxicity test guideline (OPPTS 870.3700).

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Study Citation:	Carney, EW; Thorsrud, BA; Dugard, PH; Zablotny, CL (2006). Developmental toxicity studies in Crl:CD (SD) rats following inhalation exposure to trichloroethylene and perchloroethylene Birth Defects Research, Part B: Developmental and Reproductive Toxicology, 77(5), 405-412
Data Type:	Gestational exposure study -TCE
HERO ID:	630415

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Animals were whole body exposed in 2-cubic-meter exposure chambers. Chamber airflow was maintained at approximately 450 L/min. This resulted in approximately 12-15 air changes per hour. The recommended minimum is 15 changes per hour.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Crl:CD (SD) rats (Charles River). Virgin female rats. Initial BW 218-222 g.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Housing adequately described. Room temperature and humidity were maintained within laboratory specific ranges (19–231C and 40–70% relative humidity). A 12-hr photoperiod was maintained for all animals. Food an water available ad libitum except during exposure periods.
	Metric 15:	Number per Group	High	× 1	1	27 dams/group; in accordance with guidelines
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Maternal toxicity - clinical signs, BW, feed consumption, mortality, maternal liver and kidney weights Reproductive/Devt - gravid uterine weights, # corpora lutea, uterine implants, resorptions, live/dead fetuses, fetal weight, external, skeletal, and visceral malformations/variations Although the current OECD test guideline 414 (updated in 2018) indicates that AGD should be measured in all live fetuses, the OECD TG 414 version available at the time of publication of this study was from 2001 and did not require measurement of AGD and the cited OPPTS guideline does not have that requirement.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation across groups
	Metric 18:	Sampling Adequacy	High	× 1	1	25-27 pregnant dams
	Metric 19:	Blinding of Assessors	High	× 1	1	Fetuses were examined under blind conditions; blinding not required for any endpoints by cited guidelines.
	Metric 20:	Negative Control Response	High	× 1	1	Control data reported. Historical control data discussed when needed to assess results.
Domain 6: Confounding / Variable Control						

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Study Citation:	Carney, EW; Thorsrud, BA; Dugard, PH; Zablotny, CL (2006). Developmental toxicity studies in Crl:CD (SD) rats following inhalation exposure to trichloroethylene and perchloroethylene Birth Defects Research, Part B: Developmental and Reproductive Toxicology, 77(5), 405-412
Data Type:	Gestational exposure study -TCE
HERO ID:	630415

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial BW reported; the only BW effect was a 22% decrease in BWG during from GD 6-9 at 600 ppm, no statistically significant changes in terminal BW or food consumption at any time during study. Respiratory rate not specifically mentioned, but no exposure-related clinical signs reported in dams, so bradyapnea unlikely. HSDB does show some evidence of respiratory irritation. Animal temperature should be measured to rule out bradypnea.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No mortalities, no clinical signs. Only attrition was time-mated females that were not pregnant (in all groups) that were not included in analysis.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Litter is statistical unit. Continuous data were tested in both studies for homogeneity of variance using Bartlett's test. using raw data Based on results, data were analyzed using either parametric or nonparametric tests. ANOVA followed by a Dunnett's test or a Bonferroni corrected Wilcoxon Rank sum test was used. Frequency of pre-implantation loss, resorptions per litter, resorptions per fetal population, and fetal variations and malformations were analyzed using a censored Wilcoxon test with Bonferroni's correction. In addition, pregnancy rates were analyzed using the Fisher's exact probability test with Bonferroni's correction. Fetal sex ratios were analyzed using a binomial distribution test.
	Metric 24:	Reporting of Data	High	× 2	2	All reproductive and developmental findings were reported quantitatively in tabular or graphical format. maternal body weights and food consumption reported in tables. Mortality and clinical signs reported qualitatively (no exposure-related findings)
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	Carney, EW; Thorsrud, BA; Dugard, PH; Zablotny, CL (2006). Developmental toxicity studies in Crl:CD (SD) rats following inhalation exposure to trichloroethylene and perchloroethylene Birth Defects Research, Part B: Developmental and Reproductive Toxicology, 77(5), 405-412
Data Type:	Gestational exposure study -TCE
HERO ID:	630415

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 229: **Animal toxicity evaluation results of Epstein et al 1992 for developmental study in rats (gd 6-15; dichloroacetic acid exposure) on growth (early life) and development and cardiovascular outcomes**

Study Citation:	Epstein, DL; Nolen, GA; Randall, JL; Christ, SA; Read, EJ; Stober, JA; Smith, MK (1992). Cardiopathic effects of dichloroacetate in the fetal Long-Evans rat Teratology, 46(3), 225-235
Data Type:	Developmental study in rats (GD 6-15) - dichloroacetic acid
HERO ID:	630518

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	DCA CK: DCA, a metabolite of TCE, was obtained from SIGMA and was of >99% purity
	Metric 2:	Test Substance Source	Medium	× 1	2	batch/lot number not reported; verification not reported.
	Metric 3:	Test Substance Purity	High	× 1	1	> 99% purity
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Distilled water; used combined controls Distilled water was used as vehicle control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control is not applicable for this study type
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly assigned
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	storage conditions reported; stability analytically confirmed.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	In three separate studies, pregnant rats were treated by oral intubation with DCA on selected days of gestation.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dams were dosed with 1,900 mg/kg/day DCA over days 6-8, 9-11 or 12-15 GD In a second study, in utero exposure to a singe dose of 2,400 mg/kg DCA on days 10,11,12,0r 13 In the third study, Dams were orally intubated on days 9,10,11,12 or 13 with 3,500 mg/kg DCA
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	single doses administered on different days of gestation.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	single doses administered on different days of gestation
	Metric 12:	Exposure Route and Method	High	× 1	1	gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	starting body weights not reported; maternal weights were collected on GD 0, but not reported.

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Study Citation:	Epstein, DL; Nolen, GA; Randall, JL; Christ, SA; Read, EJ; Stober, JA; Smith, MK (1992). Cardiopathic effects of dichloroacetate in the fetal Long-Evans rat Teratology, 46(3), 225-235
Data Type:	Developmental study in rats (GD 6-15) - dichloroacetic acid
HERO ID:	630518

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animals were housed in groups of two, fed with Purina rodent chow and ad libitum water. Proper animal husbandry procedures were followed
	Metric 15:	Number per Group	High	× 1	1	7-10 dams per group; number of litters and mean number of fetuses/litter were reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	morphological characterization of fetal heart defects,
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No inconsistencies were reported
	Metric 18:	Sampling Adequacy	High	× 1	1	dam and litter data provided
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable; initial histopathology evaluation
	Metric 20:	Negative Control Response	High	× 1	1	control animal response was reported
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences between study groups
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Appropriate statistical methods were used
	Metric 24:	Reporting of Data	High	× 2	2	Appropriate end points (Fetal heart development, mean heart defects) were reported
Overall Quality Determination‡			High		1.1	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 230: **Animal toxicity evaluation results of Johnson et al 1998 for a developmental study on growth (early life), development, and cardiovascular outcomes**

Study Citation:	Johnson, PD; Dawson, BV; Goldberg, SJ (1998). Cardiac teratogenicity of trichloroethylene metabolites Journal of the American College of Cardiology, 32(2), 540-545
Data Type:	developmental study - cardiac teratogenicity --metabolites
HERO ID:	630654

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Seven metabolites of TCE and DCE including TCAA, MCAA, TCEth, CMC, TCAld, DCAld, DCVC were identified
	Metric 2:	Test Substance Source	Low	× 1	3	Omitted details on the source of the test substance and/or the analytical verification of test substance; unclear if this is likely to have a substantial impact on results. Source of the test substance or analytical methods of synthesis was not provided, it is not known whether the test substance was obtained from a manufacturer
	Metric 3:	Test Substance Purity	Low	× 1	3	purity was not reported; unclear the impact on results. Purity of the test substance was not provided
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Control animals received distilled water through out the pregnancy
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for this study type
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups; unclear the impact on results.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	preparation and storage conditions reported and appropriate.Preparation of the test substance was reported, however, storage conditions were not reported
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Although the animals were consistently exposed to the chemicals through drinking water, there were four females in a pen and all received the water from the same bottle (based on what is described in the study), and the consumption of water was recorded, leading to variation in water/test substance consumption inconsistently.

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Study Citation:	Johnson, PD; Dawson, BV; Goldberg, SJ (1998). Cardiac teratogenicity of trichloroethylene metabolites Journal of the American College of Cardiology, 32(2), 540-545
Data Type:	developmental study - cardiac teratogenicity --metabolites
HERO ID:	630654

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported; water consumption was monitored and recorded and assumed to be used to calculate daily administered dose. Equivalent and actual dose was reported. water consumption appear to be calculated as an average per animal
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	animals were exposed through out pregnancy - 21 days, except for two groups where they were only exposed for 20 days due to mechanical failure of the equipment.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	only a single dose tested per metabolite; however doses of metabolites were based on the dose equivalent to that expected if all of the TCE dose (at the limit of solubility; 1100 ppm) was completely broken down the specific metabolites. Single dose exposure, also metabolite equivalent dose was based on TCE limit of solubility
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Exposure route is drinking water, however, it appears that one bottle was provided per cage that had 4 animals and to total consumption of water by all four animals was recorded every 24 hours and averaged
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Obtained from a commercial source. Characteristics sufficiently reported.

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Study Citation:	Johnson, PD; Dawson, BV; Goldberg, SJ (1998). Cardiac teratogenicity of trichloroethylene metabolites Journal of the American College of Cardiology, 32(2), 540-545
Data Type:	developmental study - cardiac teratogenicity --metabolites
HERO ID:	630654

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	" This study was conducted in Association for Assessment and Accreditation of Laboratory Animal Care accredited and Institutional Animal Care and Use Committee governed facilities at the University of Arizona Animal Care Center. Animals were quarantined for 7 days before study. Study groups consisted of virus free, young, sexually mature Hsd:Sprague Dawley SD rats (Harlan Sprague Dawley, Inc., Indianapolis, Indiana). Females, 225 6 30 g, were housed in pens of four, and the males, 300 6 50 g, were housed individually. All rats had access to water and Teklad 4% Mouse Rat diet (Teklad, Madison, Wisconsin) ad libitum. Each animal was identified by an ear notch code. The number of animals in each group was determined by a power calculation to detect a threefold increase in the malformations over controls."
	Metric 15:	Number per Group	High	× 1	1	Number of animals/group (metabolite) reported in Table 2. Number of dams and fetuses were reported
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Maternal weights, fetal position, fetal weights, gross fetal abnormalities, cardiac anomalies were recorded
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	litter and fetus data provided. Maternal rats, number of fetus (both abnormal and dead) were reported
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for initial histopathology review
	Metric 20:	Negative Control Response	Medium	× 1	2	Biological responses of negative control groups were generally adequate; though a relatively high number of atrial septal defects were found in control rats compared to treated rats.
Domain 6: Confounding / Variable Control						

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Study Citation:	Johnson, PD; Dawson, BV; Goldberg, SJ (1998). Cardiac teratogenicity of trichloroethylene metabolites Journal of the American College of Cardiology, 32(2), 540-545
Data Type:	developmental study - cardiac teratogenicity --metabolites
HERO ID:	630654

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Because it appears that the animals from each pen were drinking from the same bottle, there is a possibility of variation in the amount of chemical intake may have varied for each animal
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No notable issues
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Appropriate statistical methods seem to have been used
	Metric 24:	Reporting of Data	High	× 2	2	All necessary data was reported in a table format
Overall Quality Determination‡			~~High~~ ⟶ Low§		~~1.5~~	
Extracted			No			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Given the major concerns with the test substance purity and source, along with the other issue mentioned above, a scoring of a Low is justified, and this study should only be used to support weight of evidence."

Table 231: **Animal toxicity evaluation results of Smith et al 1989 for a teratogenicity study in rats (gd 6-15) on growth (early life) and development outcomes**

Study Citation:	Smith, MK; Randall, JL; Read, EJ; Stober, JA (1989). Teratogenic activity of trichloroacetic acid in the rat Teratology, 40(5), 445-451
Data Type:	teratogenicity study in rats GD 6-15 - trichloroacetic acid
HERO ID:	630985

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Trichloroacetic acid - TCA - metabolite of TCE
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was specified; however, the batch/lot numbers were not provided.; unlikely to have a substantial impact on results.
	Metric 3:	Test Substance Purity	High	× 1	1	> 99% purity
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	distilled water was used as vehicle control
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for this study type
	Metric 6:	Randomized Allocation	High	× 1	1	"Assignment to treatment groups was by computerized randomization such that within a block mean weights did not differ significantly"
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	test substance preparation was reported, though storage conditions were not; unlikely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Pregnant rats were orally exposed to various doses for 10 days
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	0, 330, 800, 1,200, or 1,800 mg/kg-day TCA (calculated as the free acid)
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	daily GD 6-15 (10 days)
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	4 exposure groups, although the lowest dose group (330 mg/kg) with a concurrent control was added toward the end of the study and thus was not represented in all blocks.
	Metric 12:	Exposure Route and Method	High	× 1	1	gavage (oral intubation)
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	strain, age, and sex were monitored. animals were monitored for health and weighed daily.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Although not specifically mentioned, the details of animal husbandry conditions described show that the authors have followed standard guidelines.
	Metric 15:	Number per Group	High	× 1	1	20-26 dams treated
Domain 5: Outcome Assessment						

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Study Citation:	Smith, MK; Randall, JL; Read, EJ; Stober, JA (1989). Teratogenic activity of trichloroacetic acid in the rat Teratology, 40(5), 445-451
Data Type:	teratogenicity study in rats GD 6-15 - trichloroacetic acid
HERO ID:	630985

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	maternal weights, fetal organ weights, fetal abnormalities were are measured
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No major inconsistencies observed
	Metric 18:	Sampling Adequacy	High	× 1	1	Appropriate sample sizes were used.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	this metric is not rated/applicable for this study.
	Metric 20:	Negative Control Response	High	× 1	1	Appropriate number of controls were used and there was no variation was observed within the controls
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences among the study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	One death was observed in a high dose group. Female dying prematurely were subjected to gross necropsy. Does not seem to impact the results because of the number of animals per treatment group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Appropriate statistical method was performed
	Metric 24:	Reporting of Data	High	× 2	2	All outcome measures were clearly reported
Overall Quality Determination‡			High		1.1	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 232: **Animal toxicity evaluation results of Narotsky et al 1995 for an oral gestational inhalation study on developmental outcomes**

Study Citation:	Narotsky, MG; Weller, EA; Chinchilli, VM; Kavlock, RJ (1995). Nonadditive developmental toxicity in mixtures of trichloroethylene, di(2-ethylhexyl) phthalate, and heptachlor in a 5 x 5 x 5 design Fundamental and Applied Toxicology, 27(2), 203-216
Data Type:	Oral gestational exposure study
HERO ID:	682077

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name.
	Metric 2:	Test Substance Source	Medium	× 1	2	Manufacturer was identified, but not lot. no., no analytical verification was performed.
	Metric 3:	Test Substance Purity	High	× 1	1	Reported as +99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Vehicle controls were used (corn oil).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not used in developmental studies.
	Metric 6:	Randomized Allocation	Low	× 1	3	Allocation was not described for the experiment using TCE only. Random allocation was reported for the mixtures study.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage of stock and dosing solutions was described (amber vial with teflon caps).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Gavage volume was consistent.ly administered and was not excessive.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Gavage doses were clearly reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	GD 6-15
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Doses were justified based on previous studies; 4 doses plus control.
	Metric 12:	Exposure Route and Method	High	× 1	1	Route and method (oral gavage in corn oil) were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal species , strain, age, and health status were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were described and adequate.
	Metric 15:	Number per Group	Medium	× 1	2	8-11 dams in developmental study
Domain 5: Outcome Assessment						

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Study Citation:	Narotsky, MG; Weller, EA; Chinchilli, VM; Kavlock, RJ (1995). Nonadditive developmental toxicity in mixtures of trichloroethylene, di(2-ethylhexyl) phthalate, and heptachlor in a 5 x 5 x 5 design Fundamental and Applied Toxicology, 27(2), 203-216
Data Type:	Oral gestational exposure study
HERO ID:	682077

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Limited parameters were evaluated (maternal wt. gain, dams with resorbed litters, pup wt. and viability, pups with eye defects). Viscereal and skeletal examinaitons were not performed.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Litter data were not reported for viability and eye defect data.
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported, but outcomes were objective.
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	
	Metric 24:	Reporting of Data	High	× 2	2	
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 233: **Animal toxicity evaluation results of Johnson et al 2003 for a developmental toxicity study in rats on cardiac malformation outcomes**

Study Citation:	Johnson, PD; Goldberg, SJ; Mays, MZ; Dawson, BV (2003). Threshold of trichloroethylene contamination in maternal drinking waters affecting fetal heart development in the rat Environmental Health Perspectives, 111(3,3), 289-292					
Data Type:	Developmental toxicity for cardiac malformations					
HERO ID:	700526					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	CASRN not given, but TCE has no ambiguity about form or structure
	Metric 2:	Test Substance Source	Medium	× 1	2	Johnson 2003 did not report any details about the source of TCE. The manufacturer was provided in the initial study, Dawson 1993, which is referenced, but it is unclear if the manufacturer is the same. Lot/batch was never provided.
	Metric 3:	Test Substance Purity	Medium	× 1	2	This study does not provide any analysis of test substance purity, and some uncertainty does exist since the source was not reported. Purity of the test substance is not expected to be a concern, however.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Controls were pooled from cohorts years apart using a mix of distilled and tap water. These details were not initially reported and were only later clarified through errata and other communications. It is possible that genetic drift and tap water contaminants could result in toxicological differences, but these factors are unlikely to result in the observation of false positives in treated groups relative to controls. The metric is therefore ranked low but acceptable.
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	High	× 1	1	Rats were randomized once pregnant
Domain 3: Exposure Characterization						

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Study Citation:	Johnson, PD; Goldberg, SJ; Mays, MZ; Dawson, BV (2003). Threshold of trichloroethylene contamination in maternal drinking waters affecting fetal heart development in the rat Environmental Health Perspectives, 111(3,3), 289-292
Data Type:	Developmental toxicity for cardiac malformations
HERO ID:	700526

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The study discusses making new test concentrations of TCE daily in order to provide "a more consistent concentration in the solution to compensate for the amount of hydrocarbon lost". No details were provided concerning TCE preparation or storage. Average concentration measured over 24hr was provided for each dose, with all doses demonstrating almost identical (16.5%) loss. 16.5% loss is significant but not overly concerning. The rarity of obtaining almost identical measurements across doses is worth noting. , Authors also reported a 35% reduction in the TCE concentration over a 24hr period. The estimated (nominal) dose received per animal was calculated based on this percentage is imprecise. It is unclear how closely this value matches the actual exposure.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Authors reported a 35% reduction in the TCE concentration over a 24hr period. The estimated (nominal) dose received per animal calculated based on average water consumption and this 35% reduction (due to expected degradation) is imprecise. It is unclear how closely these estimated values match the actual exposure concentration.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Exposures were calculated based on water consumption measures of group-housed animals, so variables in individual animal drinking rates are unaccounted for.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	The study reported consistent drinking water exposure throughout pregnancy for 22 days. Since pregnancy can vary slightly, it is unclear if 22 days is merely an average. There was also no discussion about how the beginning of pregnancy was indicated (e.g. observed vaginal plug).
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	This study utilized four exposure groups approximately 10-fold apart. The dose selection was adequate because Dawson 1993 served essentially as a range finding study, and two lower doses were added based on those results.

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Study Citation:	Johnson, PD; Goldberg, SJ; Mays, MZ; Dawson, BV (2003). Threshold of trichloroethylene contamination in maternal drinking waters affecting fetal heart development in the rat Environmental Health Perspectives, 111(3,3), 289-292
Data Type:	Developmental toxicity for cardiac malformations
HERO ID:	700526

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	As discussed above, the authors describe remaking TCE daily in drinking water and also provide measurements of 24hr concentrations. While there was TCE loss (16.5% in all dose groups), the consistency of these results suggest that the dose-response of the effect is reliable.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The original publication only reported the animal strain. Communication in 2014 indicates that the animals were all purchased from the same manufacturer. Communication in 2008 provides data on body weight gain for treatment groups, however data is not given for controls and a time-course is not provided. The associated Dawson 1993 study does report average body weight of females.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Housing conditions were reported however no information was provided concerning other husbandry parameters such as temperature or light-dark cycle. It is expected that all treatment groups and controls followed the same husbandry conditions, and 2014 communication with the author indicates that the same facility and researchers were used throughout the study duration.
	Metric 15:	Number per Group	Medium	× 1	2	The number of maternal rats/litters and fetuses were reported for all dose groups. Over 100 fetuses were scored for each test condition from at least 9 litters. Based on the abnormal dose response for the outcomes, increasing power by adding additional litters per group may have been useful for the study. EPA guidance recommends 20 litters/group for sufficient statistical power. Using nested BMD modeling can help partially mitigate this issue.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Detailed coding of heart defects was performed. The procedure was well described in the original publication and 2014 communication.

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Study Citation:	Johnson, PD; Goldberg, SJ; Mays, MZ; Dawson, BV (2003). Threshold of trichloroethylene contamination in maternal drinking waters affecting fetal heart development in the rat Environmental Health Perspectives, 111(3,3), 289-292
Data Type:	Developmental toxicity for cardiac malformations
HERO ID:	700526

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	The original publication failed to report that different dose groups were assessed years apart and controls were pooled from multiple experiments. The study also used drinking water (tap water) for earlier experiments and distilled water for later experiments, with controls pooled from both. These details were eventually reported in subsequent errata and published communications. Reporting was extremely poor for this study, and genetic drift over time could possibly contribute to the inconsistent dose curve. The failure to report these details initially is concerning, however outcome assessment was performed using the same processing and evaluation methods by the same researchers (per 2014 communication). Husbandry and dose administration were also otherwise the same. While the deficiencies may impact results, consistencies in the other metrics retains the metric as acceptable.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Both litter data and individual pup data were provided for the study. Only 9-13 litters were evaluated for each of the treatment groups instead of the recommended 20, and there were substantially more litters analyzed in controls.
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding was indicated in the original publication, and the 2014 communication provides additional details on the protocol. Hearts were only marked as positive for cardiac defects following unanimous agreement among three assessors.
	Metric 20:	Negative Control Response	Medium	× 1	2	It is unclear whether the variation in biological activity was impacted by the use of chlorinated tap water for some pooled controls. There were cardiac defects observed in control animals while zero defects were identified in animals at the lowest TCE dose, which is questionable. The overall rates of defects observed in control animals were reasonable, however.
Domain 6: Confounding / Variable Control						

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Study Citation:	Johnson, PD; Goldberg, SJ; Mays, MZ; Dawson, BV (2003). Threshold of trichloroethylene contamination in maternal drinking waters affecting fetal heart development in the rat Environmental Health Perspectives, 111(3,3), 289-292
Data Type:	Developmental toxicity for cardiac malformations
HERO ID:	700526

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Details on food/water intake or body weight were not provided in the paper. Body weight gain in pregnant dams was reported for treatment groups in the 2008 communication and no obvious patterns are observed, however neither a statistical analysis or time series measurement was performed. Very similar percentage losses in TCE from drinking water between groups indicate that any significant intake differences are unlikely, and confounding effects are not expected.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	There was no information provided on health outcomes unrelated to exposure, however there is no indication that any unrelated health outcomes existed. Variation introduced by the wide difference in timing among pooled controls and associated differences between test conditions for subsequent treatment groups could potentially have some effect but should not significantly impact the outcome (i.e., incidence and severity of cardiac defects).
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were adequately described.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data was presented in both tabular and graphical form for overall cardiac defects. Individual types of defects were presented in tables. While statistical significance was indicated, variance and errors bars were not shown or tabulated in graphs or tables.
Overall Quality Determination‡			Medium		1.9	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 234: **Animal toxicity evaluation results of Dawson et al. 1990 for a developmental toxicity study on growth (early life) and development outcomes**

Study Citation:	Dawson, B; Johnson, P; Goldberg, S; Ulreich, J (1990). Cardiac teratogenesis of trichloroethylene and dichloroethylene in a mammalian model Journal of the American College of Cardiology, 16(5), 1304-1309
Data Type:	Developmental toxicity
HERO ID:	701707

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified.
	Metric 2:	Test Substance Source	High	× 1	1	The test substance source was reported (Aldrich Chemical Company).
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity and grade were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The concurrent control group (vehicle only) was appropriate.
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Preparation of the test substance was reported; however, storage conditions used prior to the study or during the study were not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details on exposure administration were reported and dosing volumes were administered consistently across the study groups (via osmotic pump).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The administered doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration were reported (2-week period) and were acceptable for the study type and outcomes of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of study groups (two plus a control) were reported and considered adequate to address the purpose of the study.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The route of exposure does not meet the PECO (intrauterine instillation via pump). Non-traditional exposure routes are still acceptable for supporting WOE studies
Domain 4: Test Organism						

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Study Citation:	Dawson, B; Johnson, P; Goldberg, S; Ulreich, J (1990). Cardiac teratogenesis of trichloroethylene and dichloroethylene in a mammalian model Journal of the American College of Cardiology, 16(5), 1304-1309
Data Type:	Developmental toxicity
HERO ID:	701707

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Authors reported species, sex, strain, life stage, weight at the beginning of the study; however, animal source, starting body weight, and health status at the start of the study were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and/or if differences existed between the exposed and control groups. These deficiencies may have a substantial impact on the results.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group (10-17 maternal animals per group) was reported, appropriate for the study type and outcome analysis, and consistent with studies of the same or similar type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcomes of interest and was sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding sampling for outcomes of interest were reported. Litter data were not reported for some exposure-related outcomes (e.g., cardiac anomales show in Table 1) and percent abnormalities are calculated on group basis (Fig 1). This makes the study unusable.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	
	Metric 20:	Negative Control Response	High	× 1	1	The response of the negative control group was reported and acceptable.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in test design and procedures were reported; however, food/water intake were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis						

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Study Citation:	Dawson, B; Johnson, P; Goldberg, S; Ulreich, J (1990). Cardiac teratogenesis of trichloroethylene and dichloroethylene in a mammalian model Journal of the American College of Cardiology, 16(5), 1304-1309
Data Type:	Developmental toxicity
HERO ID:	701707

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 23: Statistical Methods	Low	× 1	3	Statistical methods were not clearly described. Some methods were mentioned in figures (e.g., Figure 2). Data were provided for some outcomes; however, data were incomplete and would not allow an independent analysis (e.g., only means, without standard deviations, were reported in Figure 1). While statistical methods for significance were not provided and the chart of offspring losses did not contain error bars, error bars were presented for cardiac defects, along with a table displaying the counts of defects. The counts of defects allow of indepenent analysis.
	Metric 24: Reporting of Data	High	× 2	2	Data for exposure-related findings were reported for each exposure group.
Overall Quality Determination‡		~~Medium~~ ⟶ Low§		~~1.7~~	
Extracted		No			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Downgraded to a low. Poor statistical reporting, however independent statistical analysis is possible. The study is still downgraded however for lack of transparency over data."

Table 235: **Animal toxicity evaluation results of Dawson et. al 1993 for a developmental study in rats on cardiovascular outcomes**

Study Citation:	Dawson, B; Johnson, P; Goldberg, S; Ulreich, J (1993). Cardiac teratogenesis of halogenated hydrocarbon-contaminated drinking water Journal of the American College of Cardiology, 21(6), 1466-1472					
Data Type:						
HERO ID:	701708					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	CASRN not given, but TCE has no ambiguity about form or structure
	Metric 2:	Test Substance Source	High	× 1	1	The paper mentions that TCE was obtained from Aldrich Chemical.
	Metric 3:	Test Substance Purity	Medium	× 1	2	This study does not provide any analysis of test substance purity. There is not a substantial concern that observed effects would be due to any impurities, however there is concern about variable concentrations across test groups.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The same negative control was used for both trichloroethylene and dichloroethylene exposure. Tap water was used as the vehicle for all animals/experimental groups. The control was not discussed in the methods section but is clearly present. Distilled water would have been a more appropriate negative control/vehicle, however the use of tap water is not expected to have any substantial impact on results, especially since it was used for all treatment groups.
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	High	× 1	1	Randomization of animals among treatment and control groups is discussed in the 2014 communication upon confirmation of insemination. The original Dawson 1993 paper does not explicitly mention randomization, but the methods described in the 2014 communication would apply to both this study and the Johnson 2003 paper.
Domain 3: Exposure Characterization						

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Study Citation:	Dawson, B; Johnson, P; Goldberg, S; Ulreich, J (1993). Cardiac teratogenesis of halogenated hydrocarbon-contaminated drinking water Journal of the American College of Cardiology, 21(6), 1466-1472
Data Type:	
HERO ID:	701708

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The study discusses preparation of diluted TCE solutions by mixing with vortex spinners covered from light exposure. Water bottles were changed daily, which is presumed to mean that solutions were made fresh daily, which would agree with the methods described in Johnson 2003. No information was provided about storage conditions for the TCE stock solution. If the data provided in Johnson 2003 for the highest two doses is presumed to apply to Dawson 1993, then average concentration measured over 24hr was provided for each dose. Both doses demonstrating almost identical (16.5%) loss. 16.5% loss is significant but not too dramatic. The rarity of obtaining almost identical measurements across doses is worth noting, however equal loss across dose groups mitigates concerns about dose-response, and may even suggest underestimation of toxicity depending on calculations. TCE loss due to volatility would be a concern for many studies, especially non-gavage. Because tap water was used for controls, it would have been useful to confirm that TCE was not detectable in control water solutions.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Drinking water administration ensures consistent exposure administration. The authors mention that the amount of water consumed was recorded, however this data was not explicitly provided.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	The Dawson 1993 manuscript itself does not report analytical measured doses. However in Johnson 2003, both initial doses and average measured dose over 24hr were reported as precise measurements. Doses were based on water consumption measures of group-housed animals, so variables in individual animal drinking rates are unaccounted for. It was also difficult to discern the details of the negative control in Dawson 1993, however the details of the negative tap water control are evident from the totality of the information provided. The total and average per day volume of consumed TCE were reported, however these values differ within exposure duration and dose groups. The basis of this measurement is unclear, but the actual delivered concentration of TCE may differ from what is reported as the estimated concentration in ppm.

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Study Citation:	Dawson, B; Johnson, P; Goldberg, S; Ulreich, J (1993). Cardiac teratogenesis of halogenated hydrocarbon-contaminated drinking water Journal of the American College of Cardiology, 21(6), 1466-1472
Data Type:	
HERO ID:	701708

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Maternal rats were exposed for either: "1) a period of approximately 2 months before pregnancy in addition to the full duration of pregnancy, 2) for the full duration of pregnancy only, and 3) an average of 3 months before pregnancy only." The beginning of pregnancy was determined by the presence of spermatozoa in vaginal smears. Dams were exposed continuously via drinking water. For a developmental toxicity study, these study durations are appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The study used only two treatment doses that were approximately 10-fold apart. The response curve indicates that these doses were of an appropriate selection, but more than two doses would have been useful. The existence of the followup study Johnson 2003 showing effects at lower doses demonstrates that additional doses would have been better. However, the included exposure levels were indeed adequate to show relevant results.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	As discussed above, the authors describe remaking TCE daily in drinking water and also provide measurements of 24hr concentrations. While there was TCE loss (16.5% in all dose groups), the consistency of these results suggest that the dose-response of the effect is reliable
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The initial publication reported the animal strain, which is appropriate for toxicology studies. The average weight and individual sex of the rats used was reported, along with the qualitative age descriptor of "young, sexually mature". Communication in 2014 indicates that the animals were all purchased from the same manufacturer. Average body weight gain is also provided for all groups.

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Study Citation:	Dawson, B; Johnson, P; Goldberg, S; Ulreich, J (1993). Cardiac teratogenesis of halogenated hydrocarbon-contaminated drinking water Journal of the American College of Cardiology, 21(6), 1466-1472
Data Type:	
HERO ID:	701708

Domain		Metric	Rating†	MWF*	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Initial housing information on the unmated female and male rats was not provided (although Johnson 2003 indicates that females were housed in groups of 3-4 and males were individually housed). Females were placed individually with a single male in a cage, however it is unclear whether the females were then separated once pregnant. No information was provided concerning other husbandry parameters such as temperature or light-dark cycle. It is expected that all treatment groups and controls followed the same husbandry conditions, and 2014 communication with the author indicates that the same facility and researchers were used throughout the study duration.
	Metric 15:	Number per Group	Medium	× 1	2	The number of exposed fetuses in each dose group were reported in Dawson 2003. The number of exposed dams/litters was not reported, however these numbers are available in both Johnson 2003 and the 2008 communication . Over 100 fetuses were scored for each test condition from at least 9 litters. Based on the abnormal dose response for the outcomes, increasing power by adding additional litters per group may have been useful for the study. Guidance typically recommends 20 litters/group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Detailed coding of heart defects was performed and well-described
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Table 1 shows the average exposure duration for each treatment group. Exposure duration varied between pre-pregnancy groups, and since averages are reported it is likely that duration differed among individual dams within each group as well. The pregnancy-only group will be the focus for data extraction and comparison with Johnson 2003. Pregnancy exposure was shorter in the 1100ppm group compared to others (18 vs 20), however it is assumed that this was due to rats giving birth earlier. Heart development is completed by GD 16 (Marcela 2012), but the study demonstrates that exposure in advance of pregnancy can possibly further promote cardiac defects so this difference cannot be ignored if the decreased pregnancy exposure time is a result of delayed exposure.

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Study Citation: Dawson, B; Johnson, P; Goldberg, S; Ulreich, J (1993). Cardiac teratogenesis of halogenated hydrocarbon-contaminated drinking water Journal of the American College of Cardiology, 21(6), 1466-1472

Data Type:

HERO ID: 701708

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Only individual pup data was provided for the study, although litter data can be calculated for the treatment groups from the data in the 2008 communication. Additionally, only 9-13 litters were evaluated for each of the treatment groups instead of the recommended 20, and there were substantially more litters analyzed in controls.
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding was indicated in Johnson 2003 and the 2014 communication, which provides additional details on the protocol. While Dawson 1993 did not explicitly mention blinding, it mentions that "decoding occurred only after final evaluation of all fetuses and hearts", presumably referring to blinded evaluation.
	Metric 20:	Negative Control Response	High	× 1	1	The negative control responses were adequate. Average weight gain was not significantly different than treatment groups, and the value of 3% abnormal hearts is reasonable for the control group.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Details on food/water intake were not provided in the paper. Body weight gain in pregnant dams was reported for all groups in Dawson 1993 and for treatment groups in the 2008 communication No obvious patterns are observed. however a statistical analysis or time-series measurement was not performed. While Johnson 2003 reported very similar losses in drinking water among groups, Table 1 in Dawson 1993 indicates that there were differences within exposure duration and dosage groups in the volume of TCE consumed. The average volume/day of TCE consumed does not appear to be consistent across groups of similar exposure duration or dose, although the values are difficult to interpret. Measured TCE concentration data in Johnson 2003 showing consistent TCE loss in all dose groups suggests that differences in air volume were not a concern for TCE loss. It is unclear how these values were measured, so it is not known if they are estimated based on differences in water intake or some other factor.

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Study Citation: Dawson, B; Johnson, P; Goldberg, S; Ulreich, J (1993). Cardiac teratogenesis of halogenated hydrocarbon-contaminated drinking water Journal of the American College of Cardiology, 21(6), 1466-1472

Data Type:

HERO ID: 701708

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	The authors state that all maternal rats were healthy throughout the study and there was no evidence of toxicity. There was also no difference between groups in terms of live births, implants, absorptions, or non-cardiac congenital abnormalities.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were adequately described.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data was presented in both tabular and graphical form for overall cardiac defects. Individual types of defects were presented in tables. Variance and errors bars were not shown or tabulated in graphs or tables, however. It was also hard to track values for the controls, as the control data was included only in the table for dichloroethylene despit being shown on the chart for TCE.
Overall Quality Determination‡			~~High~~ ⟶ Medium§		~~1.6~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Although this study is calculated as "High" and has less concerns than the Johnson 2003 followup study, there are some significant issues that prevent it from being a "High". 1) It does not report per-litter values. These are provided for the pregnancy-only treatment groups in Johnson 2003, but it cannot be calculated for controls. 2) Table 1 suggests that the actual volume of TCE consumed may differ within groups of the same exposure duration or dose, and that the ratio between doses may not be consistent. This is potentially significant for the overall dose-response determination."

Table 236: **Animal toxicity evaluation results of Fisher et al 2001 for a developmental toxicity study in rats on growth (early life) and development outcomes**

Study Citation:	Fisher, J; Channel, S; Eggers, J; Johnson, P; Macmahon, K; Goodyear, C; Sudberry, G; Warren, D; Latendresse, J; Graeter, L (2001). Trichloroethylene, trichloroacetic acid, and dichloroacetic acid: Do they affect fetal rat heart development International Journal of Toxicology, 20(5), 257-267
Data Type:	developmental toxicity study in rats (with focus on heart effects)
HERO ID:	701968

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer name and lot number were provided. It was not explicitly stated that test substance identity was verified by the manufacturer (but it is assumed).
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity of the test substance was not reported, but is not expected to be of concern.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate concurrent negative control group (soybean oil-control).
	Metric 5:	Positive Controls	High	× 1	1	Although a concurrent positive control group was not required by study type, a positive control group (administered retinoic acid) was used and responded appropriately to treatment.
	Metric 6:	Randomized Allocation	High	× 1	1	The study reported that animals were randomly allocated into study groups (using the randomization regimen in the PATH/TOX system).
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation and storage methods were reported. The stock solutions were analyzed weekly for stability.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of gavage administration were reported and were consistent across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The administered doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type (i.e., during organogenesis to evaluate effects on heart development).

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Study Citation:	Fisher, J; Channel, S; Eggers, J; Johnson, P; Macmahon, K; Goodyear, C; Sudberry, G; Warren, D; Latendresse, J; Graeter, L (2001). Trichloroethylene, trichloroacetic acid, and dichloroacetic acid: Do they affect fetal rat heart development International Journal of Toxicology, 20(5), 257-267
Data Type:	developmental toxicity study in rats (with focus on heart effects)
HERO ID:	701968

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	There was only one quantitative dose group plus the control. The study was designed to evaluate the effects of several compounds (including TCE) on heart development. Based on the nonmonotonic dose response for TCE in studies examining cardiac defects, a range of doses would have been highly preferred.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain, sex, age, and starting body weight were reported; animals were obtained from a commercial laboratory. The species is appropriate to evaluate the outcome of interest (i.e., heart defects were seen in other rat studies).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions (temperature, humidity, light/dark cycle) were inadequately reported, but are not expected to have a substantial impact on results.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group (approximately 20) was reported and considered appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported and was sensitive for the outcome of interest. The dissection methodology was overseen by Paula Johnson, author of Dawson 1993 and Johnson 2003, which observed statistically significant increases in cardiac defects following TCE administration.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups (i.e., the same time after exposure) and using the same methods.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported and were considered adequate (data were provided for per litter and per fetus incidences).
	Metric 19:	Blinding of Assessors	High	× 1	1	The study explicitly reported that all determinations were made "blindly." In addition, all members of the cardiac dissection team utilized the same stepwise dissection and examination protocol.

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Study Citation:	Fisher, J; Channel, S; Eggers, J; Johnson, P; Macmahon, K; Goodyear, C; Sudberry, G; Warren, D; Latendresse, J; Graeter, L (2001). Trichloroethylene, trichloroacetic acid, and dichloroacetic acid: Do they affect fetal rat heart development International Journal of Toxicology, 20(5), 257-267
Data Type:	developmental toxicity study in rats (with focus on heart effects)
HERO ID:	701968

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Negative Control Response	Low	× 1	3	The biological responses of the control group were reported. However, the soybean oil control group produced a higher incidence of heart defects than the TCE-treated group, which produced a much higher incidence than the water control. The high background incidence of fetal heart malformations may have impacted the study results. The incidence of cardiovascular effects was much greater be higher in soybean oil controls. leading to no significant differences between the control and the TCE treatment group for combined heart and cardiovascular effects (despite a ~20% higher per litter incidence of cardiac-specific effects in TCE vs soybean oil control).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among study groups (with respect to initial body weights, food consumption, etc.) that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	No data on attrition or outcomes unrelated to exposure were reported; only substantial differences among groups were noted (not likely to impact results).
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical methods were well-described and appropriate for the datasets of interest. It would have been more informative if statistical significance was additionally tested for cardiac-specific and cardiovascular effects independently in addition to combined cardiac and cardiovascular because of the high negative control incidence in cardiovascular effects, which was much lower than TCE for cardiac effects but was higher for cardiovascular effects.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were adequately reported for all outcomes by exposure group.
Overall Quality Determination‡			~~High~~ ⟶ Medium§		~~1.3~~	
Extracted			Yes			

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Study Citation:	Fisher, J; Channel, S; Eggers, J; Johnson, P; Macmahon, K; Goodyear, C; Sudberry, G; Warren, D; Latendresse, J; Graeter, L (2001). Trichloroethylene, trichloroacetic acid, and dichloroacetic acid: Do they affect fetal rat heart development International Journal of Toxicology, 20(5), 257-267
Data Type:	developmental toxicity study in rats (with focus on heart effects)
HERO ID:	701968

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study
§ Evaluator's explanation for rating change: "Downgraded due to major issues with the negative control incidence, which showed effects in over 50

Table 237: **Animal toxicity evaluation results of Forkert et al 2002 for a 4-wk inhalation study in mice on reproductive, growth (early life) and developmental outcomes**

Study Citation:	Forkert, P; Lash, L; Nadeau, V; Tardif, R; Simmonds, A (2002). Metabolism and toxicity of trichloroethylene in epididymis and testis Toxicology and Applied Pharmacology, 182(3), 244-254
Data Type:	4 week inhalation study of sperm effects in mice
HERO ID:	701988

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained from commercial source
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance reported to be 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Study references a concurrent negative control group but does not describe whether this group was untreated or sham-treated, nor is it clear whether or not the exposed mice were restrained during exposure.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not typical for male repro tox
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Method for generation of test substance was described, but study did not report storage of test material.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Study does not clearly indicate whether exposures were whole-body or nose-only. However, based on the lack of information on restraint methods, it is likely that animals were exposed whole-body.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Actual concentration of gas was not reported but there is high confidence that exposures were close to targeted as air concentrations were verified by GC every 4 minutes on the first and fourth days of the week.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Mice were exposed 6 hr/d, 5 d/wk for 4 wks (total of 19 exposures). This frequency is typical but the duration is shorter than subchronic and longer than acute.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only a single exposed group was tested.

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Study Citation: Forkert, P; Lash, L; Nadeau, V; Tardif, R; Simmonds, A (2002). Metabolism and toxicity of trichloroethylene in epididymis and testis Toxicology and Applied Pharmacology, 182(3), 244-254
Data Type: 4 week inhalation study of sperm effects in mice
HERO ID: 701988

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Animals were exposed by inhalation; study did not indicate whether nose-only or whole-body, but lack of information on restraint for nose-only administration suggests that exposure was whole-body.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain, sex, and body weight were reported, but age was not.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Few animal husbandry conditions were reported (light dark cycle only); no information on temperature, humidity, or housing was reported.
	Metric 15:	Number per Group	Medium	× 1	2	Group sizes were smaller than typical but sufficient for statistical analysis (6)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Toxicological evaluations were limited to histopathology of the testes and epididymides.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Study lacks information on timing and methods of sacrifice for the histopathology experiment (provided for ADME and mechanistic evaluations).
	Metric 18:	Sampling Adequacy	Low	× 1	3	Study does not specify numbers of animals examined for histopathology
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not typical for initial histopathology review
	Metric 20:	Negative Control Response	Low	× 1	3	Apart from a pathology slide, no information on control response was reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Study did not report any differences between control and exposed group that could impact results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	No health outcomes unrelated to exposure were reported., but few details were given for the histopathology experiment.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	Statistical analysis was not performed/reported and data enabling independent statistical analysis were also not reported.
	Metric 24:	Reporting of Data	Low	× 2	6	Histopathology results were described only qualitatively and only for the exposure group and not controls.
Overall Quality Determination‡			Unacceptable⋆⋆		2.3	

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Study Citation:	Forkert, P; Lash, L; Nadeau, V; Tardif, R; Simmonds, A (2002). Metabolism and toxicity of trichloroethylene in epididymis and testis Toxicology and Applied Pharmacology, 182(3), 244-254
Data Type:	4 week inhalation study of sperm effects in mice
HERO ID:	701988

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		No			

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 238: **Animal toxicity evaluation results of Isaacson et al 1989 for a drinking water exposure study during gestation in rats on growth (early life) and development outcomes**

Study Citation:	Isaacson, LG; Taylor, DH (1989). Maternal exposure to 1,1,2-trichloroethylene affects myelin in the hippocampal formation of the developing rat Brain Research, 488(1-2), 403-407
Data Type:	Study of drinking water exposure during gestation on myelin in brain of rat offspring
HERO ID:	704481

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test material identified by unambiguous name
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance source was reported but without batch/lot number and/or certified/analytically verified identity.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was not reported. CK: Given reference for the chemical information source - (Mallinckrodt)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative control group was exposed to distilled water.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control is not typical for this study type.
	Metric 6:	Randomized Allocation	High	× 1	1	Study reports random allocation of maternal animals to test groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage of test substance were described and appeared to be adequate; steps were taken to minimize volatilization during drinking water preparation, and TCE degradation between test material preparations was accounted for..
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were reported, but information on drinking water intake rates by exposure group were not reported so it is unclear whether there were any palatability concerns.

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Study Citation:	Isaacson, LG; Taylor, DH (1989). Maternal exposure to 1,1,2-trichloroethylene affects myelin in the hippocampal formation of the developing rat Brain Research, 488(1-2), 403-407
Data Type:	Study of drinking water exposure during gestation on myelin in brain of rat offspring
HERO ID:	704481

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Doses were reported in terms of mg/day and were based on average water intake during exposure, but it is not clear which groups' intakes were included in the average. Maternal body weights were not reported, so dose per body weight estimates will be uncertain. CK: Drinking water was changed every 24 h. Daily dosages of TCE received by the dams were calculated using previously determined degradation data for TCE over a 24 h period 23 and average daily intake of TCE/water (approximately 27 ml) by the dams throughout the exposure period. Thus, dams' drinking water which was mixed initially in concentrations of 312 or 625 mg/liter received average doses of approximately 4.0 or 8.1 rag/day of TCE, respectively, for 56 days. The amount of TCE and/or its degradation products to which the pups were exposed while in utero and during lactation was approximated using a combination of blood plasma measurements and predictions from mathematical models developed specifically for this purpose 4"5. Rat pups whose dams received 4.0 mg/day TCE received a daily dose of 0.003 /,g/ml TCE, 0.100 ~g/ml trichloroethanol, and 1.9 j,g/ml trichloroacetic acid. Pups whose dams received a daily dose of 8.1 mg/day TCE were exposed to 0.012 /~g/ml TCE, 0.320 /ag/ml trichloroethanol, and 3.5 ug/ml trichloroacetic acid.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	The exposure frequency was not reported, but because it is a drinking water study it is reasonable to assume the animals were exposed 7 days/week. The duration was adequate for the outcome of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Two nonzero exposure concentrations were administered; these differed by 2 fold. Doses were adequate to observe an effect, but a NOEL was not identified.
	Metric 12:	Exposure Route and Method	High	× 1	1	Route and method of exposure were reported and appropriate to the substance
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Source, strain, sex, and age were reported and appropriate; however initial body weights were not given.

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Study Citation:	Isaacson, LG; Taylor, DH (1989). Maternal exposure to 1,1,2-trichloroethylene affects myelin in the hippocampal formation of the developing rat Brain Research, 488(1-2), 403-407
Data Type:	Study of drinking water exposure during gestation on myelin in brain of rat offspring
HERO ID:	704481

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	No animal husbandry conditions were reported. CK: Animals were housed in the Miami University animal facilities with food and water available ad libitum. Assumed that University animal facilities must be maintained with adequate animal husbandry conditions.
	Metric 15:	Number per Group	Unacceptable	× 1	4	Study reports that six female rats were allocated to 3 groups, leaving only 2 dams per group. This number of dams is insufficient to characterize toxicological effects.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The only outcome evaluated was myelin levels in the brains of offspring. While sensitive, this endpoint provides a limited evaluation of neurotoxicity. The methodology for outcome assessment was provided in detail.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	There were no reported differences in outcome assessment across groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Outcomes were assessed in male offspring only, and from 3-4 hippocampal sections of 2-3 animals per treatment group. The litter distribution of the animals was not reported.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Outcome was not subjective.
	Metric 20:	Negative Control Response	Medium	× 1	2	Most control responses were described qualitatively but this is not expected to influence the results.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food and water intake (except for a single average intake estimate without specification of dose group[s]) were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	No information on attrition or health outcomes apart from the primary outcome was provided.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical analysis methods were reported, but the study does not indicate whether the litter was the unit of statistical analysis.

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Study Citation:	Isaacson, LG; Taylor, DH (1989). Maternal exposure to 1,1,2-trichloroethylene affects myelin in the hippocampal formation of the developing rat Brain Research, 488(1-2), 403-407
Data Type:	Study of drinking water exposure during gestation on myelin in brain of rat offspring
HERO ID:	704481

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	Low	× 2	6	Most results were reported qualitatively and with photomicrographs; mean numbers of myelinated fibers were reported without a measure of variability.
Overall Quality Determination‡		Unacceptable⋆⋆		2.0	
Extracted		No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 239: **Animal toxicity evaluation results of Hardin et al 1981 for a developmental toxicity study on growth (early life) and development outcomes**

Study Citation:	Hardin, BD; Bond, GP; Sikov, MR; Andrew, FD; Beliles, RP; Niemeier, RW (1981). Testing of selected workplace chemicals for teratogenic potential Scandinavian Journal of Work, Environment and Health, 7(Suppl 4), 66-75
Data Type:	Developmental toxicity
HERO ID:	62211

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Neither purity nor grade were reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Controls were used but specifics, including how they were exposed, were not reported.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not required.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage were not reported and there was no mention of the method and equipment used to generate the test substance.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Details on exposure administration were not reported so it is unknown if methods were consistent among the groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Actual inhalation exposure concentrations were not reported.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Exposure frequency and duration are not clearly reported for TCE exposures. Other chemicals were tested and the report states "in most cases, the animals were exposed for 6 to 7 h/d on gestation days 1 to 19 (rats) or 1 to 24 (rabbits), but exceptions, if they occurred, were not stated.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only one quantitative exposure group, 500 ppm, was tested, and a control. A single dose group does not exclude the utility of the study for qualitative and WOE assessment.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The route was reported, however, it was not stated whether the exposures were nose- or head-only, or whole body. Air changes per hour were not reported.
Domain 4: Test Organism						

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Study Citation:	Hardin, BD; Bond, GP; Sikov, MR; Andrew, FD; Beliles, RP; Niemeier, RW (1981). Testing of selected workplace chemicals for teratogenic potential Scandinavian Journal of Work, Environment and Health, 7(Suppl 4), 66-75
Data Type:	Developmental toxicity
HERO ID:	62211

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Beyond species (rats, rabbits) no other test animal characteristics were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and/or if differences existed between the exposed and control groups.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals was not clearly reported, other than the authors noting in the methods section that the target number of litters was 30 for rats and 20 for rabbits.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Due to incomplete reporting, it is unclear whether methods were sensitive for the outcome of interest. Endpoints evaluated were not fully reported.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Details regarding the execution of the study protocol for outcome assessment were not reported and this may have a substantial impact on the results.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding sampling of outcomes were not reported and this deficiency is likely to have a substantial impact on results.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No evaluations that were considered subjective were conducted, so this metric is not applicable.
	Metric 20:	Negative Control Response	Unacceptable	× 1	4	Biological responses of the negative control groups were not reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food/water intake, and respiratory rate were not reported. These deficiencies are likely to have a substantial impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical methods were not clearly described for TCE tests.
	Metric 24:	Reporting of Data	Low	× 2	6	Data for exposure-related findings were not shown for each study group but results were briefly described in the text. This is likely to have a substantial impact on results.
Overall Quality Determination‡			Unacceptable⋆⋆		2.8	

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Study Citation:	Hardin, BD; Bond, GP; Sikov, MR; Andrew, FD; Beliles, RP; Niemeier, RW (1981). Testing of selected workplace chemicals for teratogenic potential Scandinavian Journal of Work, Environment and Health, 7(Suppl 4), 66-75
Data Type:	Developmental toxicity
HERO ID:	62211

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		No			

** Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 240: **Animal toxicity evaluation results of Kumar et al 2001 for a subchronic inhalation study in rats on reproductive, endocrine, and spermatogenesis outcomes**

Study Citation:	Kumar, P; Prasad, A; Mani, U; Maji, B; Dutta, K (2001). Trichloroethylene induced testicular toxicity in rats exposed by inhalation Human & Experimental Toxicology, 20(11), 585-589
Data Type:	Subchronic inhalation study
HERO ID:	706576

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name
	Metric 2:	Test Substance Source	Medium	× 1	2	Manufacturer identified, but not lot number.
	Metric 3:	Test Substance Purity	High	× 1	1	99% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Age-matched male rats exposed to compressed air
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not used for this study type.
	Metric 6:	Randomized Allocation	High	× 1	1	Randomly divided into groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Exposure details were reported in other publications. CK: Not Sure
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Target concentration was not reported. Concentration was measured by HPLC with UV-visible detector. 8 samples during 4 hrs (not throughout the exposure duration).
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	4 h/day instead of 6 h/day. Duration of 12 and 24 weeks
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only 1 concentration was used.
	Metric 12:	Exposure Route and Method	Not Rated	NA	NA	Details regarding exposure (ie., air excahnges, condensation) are provided in other papers.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain, commercial source and age were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Describe well
	Metric 15:	Number per Group	Medium	× 1	2	6/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Testes wt., spermatogenesis, sperm count and motility, histopath., testicular marker enzymes.

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Study Citation:	Kumar, P; Prasad, A; Mani, U; Maji, B; Dutta, K (2001). Trichloroethylene induced testicular toxicity in rats exposed by inhalation Human & Experimental Toxicology, 20(11), 585-589
Data Type:	Subchronic inhalation study
HERO ID:	706576

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding not reported, but outcomes were objective,
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not reported; expected to be an irritant.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No deaths were recorded.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	
	Metric 24:	Reporting of Data	High	× 2	2	
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 241: **Animal toxicity evaluation results of Peden-Adams et al 2006 for a developmental immunotoxicity study in mice on growth (early life) and developmental outcomes**

Study Citation:	Peden-Adams, MM; Eudaly, JG; Heesemann, LM; Smythe, J; Miller, J; Gilkeson, GS; Keil, DE (2006). Developmental immunotoxicity of trichloroethylene (TCE): Studies in B6C3F1 mice Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 41(3), 249-271
Data Type:	Developmental Immunotox study
HERO ID:	707381

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE is not ambiguous
	Metric 2:	Test Substance Source	High	× 1	1	Sigma; batch not reported. Batch not required because TCE does not vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	Not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Carrier control (1%emulphor)
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed for study type
	Metric 6:	Randomized Allocation	High	× 1	1	Parental mice (five C3H males and five C57Bl6 females randomly assigned and separated into pairs within each treatment group)
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Emulphor (1% solution) was used as a vehicle to increase solubility and maintain uniform distribution of the TCE in the drinking water carrier. Drinking water solutions were changed every other day. Storage conditions not mentioned. Downgraded to medium.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Consistent between groups. Analytical tests conducted by General Engineering (Charleston, SC) confirmed TCE concentrations were consistently maintained in the drinking water during exposure.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Target concentrations reported in ppb. Water intake levels not reported, so actual doses cannot be determined from reported data. Pup body weight data reported at 3 wk and 8 wk; parental BW not reported. Default BW and intake values would need to be used to estimate parental dosing during mating and maternal dosing through gestation and lactation. For pups, reported BW and allometric scaling could be used to estimate pup dosing in the group that was exposed form PNW3-8.

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Study Citation:	Peden-Adams, MM; Eudaly, JG; Heesemann, LM; Smythe, J; Miller, J; Gilkeson, GS; Keil, DE (2006). Developmental immunotoxicity of trichloroethylene (TCE): Studies in B6C3F1 mice Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 41(3), 249-271
Data Type:	Developmental Immunotox study
HERO ID:	707381

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	GD 0 - PNW 3 (via dam); some pups continued direct dosing from PNW 3-8. Drinking water available ad libitum.; parental animals were exposed for 2 wks during mating as well. Exposure post-weaning is not typical but is not inappropriate for the outcomes examined.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 exposure levels plus control. Effects were seen at the lowest dose, and only two doses were used. A third lower dose would have been useful.
	Metric 12:	Exposure Route and Method	High	× 1	1	Drinking water, mixed every other day and concentration was verified.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Parental animals were adult female C57BL/6N mice and adult C3H/HeJ male mice (Harlan-Teklab). Pups generated from this mating were B6C3F1, a common strain used in immunotoxicology testing. Parental BWs not reported. Age also not reported
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Room conditions and housing reported. Food and water were available ad libitum.
	Metric 15:	Number per Group	Medium	× 1	2	5 mating pairs/group; Methods states that 5-7 pups/group assessed at PNW 3, remaining pups/group continued direct treatment until assessment at PNW 8. Based on data presentation, it appears that 5-7/sex/group were assessed at each time point (total pup number was 9-14 pups/group with sexes combined). No discussion of litter distribution in each group. Ideally, 1 pup/sex/litter would have been assessed at each time-point so the litter could be the statistical unit. But the number of litters is low. According to OECD 443 (Extended One-Gen Repro Tox), 20 pups/group (10M and 10F), each representing a different litter, is appropriate. However, the number used was sufficient for statistical analysis.
Domain 5: Outcome Assessment						

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Study Citation:	Peden-Adams, MM; Eudaly, JG; Heesemann, LM; Smythe, J; Miller, J; Gilkeson, GS; Keil, DE (2006). Developmental immunotoxicity of trichloroethylene (TCE): Studies in B6C3F1 mice Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 41(3), 249-271
Data Type:	Developmental Immunotox study
HERO ID:	707381

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Immune outcomes, body weight, and immune organ and hepatic and kidney weight were evaluated at PNW 3 and PNW 8 in offspring. Exposure was pre-mating through gestation and lactation (with some offspring exposed post-weaning). No assessment of parental toxicity or neonatal toxicity (litter parameters, birth weights, gross observations, etc). These omissions greatly impact interpretation of results at PNW 3 and PNW 8 since general health conditions of parental animals and neonates are unknown.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Assessed consistently across groups. However, the litter distribution of evaluated pups was not reported. If distribution was not even (e.g. majority of pups evaluated at a specific dose were from one litter), that could affect consistency. However, this issue is addressed in Metric 18, not here.
	Metric 18:	Sampling Adequacy	Low	× 1	3	There were 5-7/sex/group evaluated for most endpoints; However, It the litter distribution of evaluated pups was not reported. There should be 1 pups/sex/litter in this type of study design for proper sampling. If all pups only came from 1 or 2 litters, that could skew results. The authors did report that each experiment was repeated at least twice, indicating technical replicates. Litters not pups also are the correct statistical unit.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No necessary for outcomes evaluated.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control data reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Iniital BW (parental) not reported. Drinking water and food intake not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						

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Study Citation:	Peden-Adams, MM; Eudaly, JG; Heesemann, LM; Smythe, J; Miller, J; Gilkeson, GS; Keil, DE (2006). Developmental immunotoxicity of trichloroethylene (TCE): Studies in B6C3F1 mice Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 41(3), 249-271
Data Type:	Developmental Immunotox study
HERO ID:	707381

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 23: Statistical Methods	Low	× 1	3	Pup was the statistical unit of exposure. For PNW 3 assessment, and potentially the PNW 8 assessment, the litter would have been the appropriate unit. Data were tested for normality (Shapiro-Wilks) and homogeneity (Bartlett's) and if needed appropriate transformations were made. Statistical significance was determined using a one-way ANOVA (p=0.05). When significant differences were detected by ANOVA, Dunnett's Comparison was used to compare treatment groups and controls. If significant gender by treatment (gender*treatment) or gender within treatment (gender[treatment]) differences were observed (p=0.05), data were separated by gender for analysis
	Metric 24: Reporting of Data	High	× 2	2	Body mass, length, and organ weights reported quantitatively. Immune endpoints with effects were reported in tables or graphically.
Overall Quality Determination‡		~~Medium~~ ⟶ Low§		~~1.8~~	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "The study was downgraded due to the following: 1) Actual doses were not reported, and cannot be calculated due to lack of parental body weight data and lack of water intake data (metric 9); 2) Concerns over sampling (lack of litter distribution data within groups); 3) Use of pup as statistical unit; and 4)Lack of general health assessment in parental animals and neonates"

Table 242: **Animal toxicity evaluation results of Smith et al 1992 for a developmental study in rats on growth (early life) and developmental outcomes**

Study Citation:	Smith, MK; Randall, JL; Read, EJ; Stober, JA (1992). Developmental toxicity of dichloroacetate in the rat Teratology, 46(3), 217-223
Data Type:	developmental study in rats GD 6-15 - dichloroacetate
HERO ID:	707968

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	dichloroacetic acid (metabolite of TCE)
	Metric 2:	Test Substance Source	Medium	× 1	2	Source was reported, but batch/lot number was not. Not likely to have a substantial impact on results.
	Metric 3:	Test Substance Purity	High	× 1	1	> 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	distilled water
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for this study type
	Metric 6:	Randomized Allocation	High	× 1	1	A random block design was used so that mean weights did not differ significantly within a block
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	test substance preparation was reported, solutions prepared daily
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Study 1: 0, 900, 1,400, 1,900, or 2,400 mg/kg-day; Study 2: 0, 14, 140, and 400 mg/kg/day; dose calculated as the free acid
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	daily GD 6-15
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	
	Metric 12:	Exposure Route and Method	High	× 1	1	gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	strain, age, and sex were monitored. animals were monitored for health and weighed at beginning of gestation
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	
	Metric 15:	Number per Group	High	× 1	1	groups of about 20 rats each
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	appropriate methodology was reported
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No notable inconsistencies were observed
	Metric 18:	Sampling Adequacy	High	× 1	1	

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Study Citation:	Smith, MK; Randall, JL; Read, EJ; Stober, JA (1992). Developmental toxicity of dichloroacetate in the rat Teratology, 46(3), 217-223
Data Type:	developmental study in rats GD 6-15 - dichloroacetate
HERO ID:	707968

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	this metric is not rated/applicable for this study.
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the negative control groups were adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences among the study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No attrition was reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Adequate statistical methods were reported
	Metric 24:	Reporting of Data	High	× 2	2	All endpoints studied were reported adequately
Overall Quality Determination‡			High		1.0	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 243: **Animal toxicity evaluation results of Xu et al 2004 for a male reproductive study in mice on body weight and reproductive outcomes**

Study Citation:	Xu, H; Tanphaichitr, N; Forkert, PG; Anupriwan, A; Weerachatyanukul, W; Vincent, R; Leader, A; Wade, MG (2004). Exposure to trichloroethylene and its metabolites causes impairment of sperm fertilizing ability in mice Toxicological Sciences, 82(2), 590-597
Data Type:	Male repro study
HERO ID:	708487

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	spectrophotometric grade TCE
	Metric 2:	Test Substance Source	Medium	× 1	2	Aldrich Chemical; lot/batch no not reported
	Metric 3:	Test Substance Purity	High	× 1	1	>99.5%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent chamber controls for each duration
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not required for study type
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The atmospheres were generated by evaporating TCE through a glass evaporative system, with the resulting vapor being carried by an air stream into the chamber inlet and mixed with the incoming air.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Concentrations of TCE in air from both chambers (i.e., TCE and control) and from the surrounding room were monitored every 6 min throughout the exposure period by gas chromatography.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Only target concentrations reported, but concentrations were monitored.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	6 hr/d, 5 d/wk for 1, 2, 4, or 6 wks.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only one exposure group plus control. (PECO states that at least 2 exposure groups should be used).
	Metric 12:	Exposure Route and Method	High	× 1	1	Whole-body, dynamic inhalation chamber (2.5 cubic meters). With a reported air flow of 500 L/min, there would be 12 air changes/hour.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Male CD-1 mice (Charles rive) 80-90 d at star of exposure. Sexually mature female CF-1 mice (Charles River) were obtained for mating study (females were not exposed). Initial BW not reported.

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Study Citation:	Xu, H; Tanphaichitr, N; Forkert, PG; Anupriwan, A; Weerachatyanukul, W; Vincent, R; Leader, A; Wade, MG (2004). Exposure to trichloroethylene and its metabolites causes impairment of sperm fertilizing ability in mice Toxicological Sciences, 82(2), 590-597
Data Type:	Male repro study
HERO ID:	708487

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Cage details and light/dark info provided. No information on temperature or humidity (but followed animal treatment guidelines).
	Metric 15:	Number per Group	High	× 1	1	4-6/group for 1 or 6 wk, 22-27/group for 2 wk, 10-15/wk for 4 wk
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Body weight, testis and epididymis weight, and sperm motility and number at each timepoint. Mating with unexposed female to determine egg fertilization rate at 2, 4, or 6 wks.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	3-15/group for in vivo fertility; 4-27/group for other endpoints
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective endpoints.
	Metric 20:	Negative Control Response	High	× 1	1	Control data reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial BW not reported. No changes in BW observed. Respiratory rate not reported, but TCE causes little to no respiratory irritation at anesthetic levels (HSDB), so bradypnea not expected.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Fertilization data: 2-factorial ANOVA (treatment, duration). Significant differences indicated by ANOVA were further evaluated by Dunnett's method Statistics were not specifically reported for other endpoints, but results were reported as "did not result in significant changes", suggesting that statistical analyses were conducted. Data reporting adequate for independent analysis.
	Metric 24:	Reporting of Data	High	× 2	2	Data reported quantitatively in table or graphical display.
Overall Quality Determination‡			High		1.4	

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Study Citation:	Xu, H; Tanphaichitr, N; Forkert, PG; Anupriwan, A; Weerachatyanukul, W; Vincent, R; Leader, A; Wade, MG (2004). Exposure to trichloroethylene and its metabolites causes impairment of sperm fertilizing ability in mice Toxicological Sciences, 82(2), 590-597
Data Type:	Male repro study
HERO ID:	708487

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i \left(\text{Metric Score}_i \times \text{MWF}_i\right) / \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 244: **Animal toxicity evaluation results of George et al 1986 for a continuous breeding study in rats on reproductive, growth (early life) and development, neurological/behavior, renal, and hepatic outcomes**

Study Citation:	George, JD; Reel, , JR; Myers, CB; Lawton, AD; Lamb, JC (1986). Trichloroethylene: Reproduction and fertility assessment in F344 rats when administered in the feed NTP 86 312 PP
Data Type:	Continuous Breeding study - Tasks 2 - 4
HERO ID:	723905

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name, CASRN, molecular weight and structure in protocol and dose analysis reports
	Metric 2:	Test Substance Source	High	× 1	1	Source, lot, and batch reported in dose analysis report
	Metric 3:	Test Substance Purity	Medium	× 1	2	Not reported, but identified as "High Purity grade" CK: Please Check on Page- 184
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle (chow) controls for each task
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed per study design
	Metric 6:	Randomized Allocation	High	× 1	1	For Task 2 - Matched by weight and randomly assigned into treatment groups. Weight-matching introduces non-random component. Animals for Task 3 and 4 were taken from the exposure groups set-up in Task 2. CK: Check on page-146, section 5.0
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	TCE was microencapsulated (70% gelatin, 29.8 % sorbital) for stability. Formulated feed sample stored in dark at 4 degrees C. New batches mixed weekly.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Task 2: Feed analysis showed that at weeks 1 and 6, the actual concentration of TCE in the feed ranged from 27-33%, 66-71%, and 82-87% at the .15%, 0.30%, and 0.60% formulations, respectively. Samples of formulations from each of the dose levels administered during week 12 and 18 of Task 2 assayed at 101-114% of the theoretical concentration.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	only target doses reported. However, initial, weekly, and final BW, daily feed consumption, and analysis of actual concentration of TCE in feed were reported, so actual compound consumption can be calculated.

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Study Citation:	George, JD; Reel, , JR; Myers, CB; Lawton, AD; Lamb, JC (1986). Trichloroethylene: Reproduction and fertility assessment in F344 rats when administered in the feed NTP 86 312 PP
Data Type:	Continuous Breeding study - Tasks 2 - 4
HERO ID:	723905

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Standard exposure protocol for Con't breeding (7 days prior to cohabitation, 98-d cohabitation, 28 d segregation).
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	dietary levels of 0.0, 0.15, 0.30, and 0.60% TCE were selected for the continuous breeding phase based on acute toxicity results from Task 1.
	Metric 12:	Exposure Route and Method	High	× 1	1	Mixed in diet, encapsulated for stability. Mixed fresh weekly.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	F344 rats (Charles River), 11 wks at start of Task 2. Initial BW reported. All evaluated for presence of 11 common viruses during quarantine.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Detailed husbandry in protocol document.
	Metric 15:	Number per Group	High	× 1	1	40/sex in control; 20/sex in exposure groups.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Comprehensive evaluation of reproductive endpoints in Task 2. Task 3 set up to determine affected sex (control and high-dose animals only). Task 4 evaluated F1 reproduction and neurobehavior. In addition to reproductive endpoints, liver, kidney, and adrenal weights and histology were evaluated in F0 animals from Task 3 and F1 animals from Task 2/4. Body weights evaluated throughout.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Consistent evaluation across defined groups. However, based on study design, organ weights in F0 animals were only evaluated in control and high-dose animals. Some significant changes were observed, so evaluating lower-dose animals may be warranted. however, no exposure-related histopathological changes were noted at the high dose, so organ weight changes may not be biologically relevant.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling according to protocol and adequate numbers.

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Study Citation:	George, JD; Reel, , JR; Myers, CB; Lawton, AD; Lamb, JC (1986). Trichloroethylene: Reproduction and fertility assessment in F344 rats when administered in the feed NTP 86 312 PP
Data Type:	Continuous Breeding study - Tasks 2 - 4
HERO ID:	723905

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 19:	Blinding of Assessors	High	× 1	1	Each formulation will be labeled with the date of preparation, the study code number, a random five-digit concentration code number and a unique color code to insure that the study is conducted blind for dose. Animal cage cards will be similarly labeled with the study code number, concentration code number and color code. Personnel involved in animal dosing, animal care, and toxicologic and reproduction evaluations will not be informed of the formulation concentrations until such time as all laboratory .work has been completed.
	Metric 20:	Negative Control Response	High	× 1	1	Control data reported. No deviations from expected.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Body weight and food consumption data reported. Adult body weight changes <20%. No reported changes in food consumption.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No mortality or clinical signs. Some histological observations were observed in liver and kidney, but were approximately equal between groups. Kidney lesions (tubular regeneration and tubular casts) were attributed to early signs of chronic progressive nephropathy that occur spontaneously in F344 rats. Hemosiderin pigment in macrophages in the stroma of the enodmetrium and myometrium of rats is commonly observed in postpartum uterus. These were not considered related to treatment.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Detailed statistical report included. Histology not evaluated statistically, but data reporting adequate for independent analysis.
	Metric 24:	Reporting of Data	High	× 2	2	Summary and individual animal data reported.
Overall Quality Determination‡			High		1.1	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study.

Table 245: **Animal toxicity evaluation results of George et al 1986 for a continuous breeding study in rats on nutrition and metabolic/adult exposure body weight and mortality outcomes**

Study Citation:	George, JD; Reel, , JR; Myers, CB; Lawton, AD; Lamb, JC (1986). Trichloroethylene: Reproduction and fertility assessment in F344 rats when administered in the feed NTP 86 312 PP
Data Type:	Continuous Breeding study - Task 1
HERO ID:	723905

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name, CASRN, molecular weight and structure in protocol and dose analysis reports
	Metric 2:	Test Substance Source	High	× 1	1	Source, lot, and batch reported in dose analysis report
	Metric 3:	Test Substance Purity	High	× 1	1	Not reported, but identified as "High Purity grade" CK: Check on page-184
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle (chow) controls
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed per study design
	Metric 6:	Randomized Allocation	Medium	× 1	2	Rats were sorted into treatment groups by weight. CK: On page 146: During the two to five week quarantine period animals will be randomly assigned to cages, individually weighed and individually coded tags will be affixed to one ear of each mouse
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	TCE was microencapsulated (70% gelatin, 29.8 % sorbital) for stability. Formulated feed sample stored in dark at 4 degrees C. New batches mixed weekly.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Analysis of the Task 1 feed formulations indicated that they ranged from 96% to 111% of the desired TCE concentrations
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	only target doses reported. However, initial and final BW, daily feed consumption, and analysis of actual concentration of TCE in feed were reported, so actual compound consumption can be calculated.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	14-day dose-range finding study.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	5 doses plus control
	Metric 12:	Exposure Route and Method	High	× 1	1	Mixed in diet, encapsulated for stability. Mixed fresh weekly.
Domain 4: Test Organism						

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Study Citation:	George, JD; Reel, , JR; Myers, CB; Lawton, AD; Lamb, JC (1986). Trichloroethylene: Reproduction and fertility assessment in F344 rats when administered in the feed NTP 86 312 PP
Data Type:	Continuous Breeding study - Task 1
HERO ID:	723905

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	High	× 2	2	F344 rats (Charles River), 8 wks at start of Task 1. Initial BW reported. All evaluated for presence of 11 common viruses during quarantine.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Detailed husbandry in protocol document.
	Metric 15:	Number per Group	High	× 1	1	8/sex/group; adequate for dose-range finding
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Assessed for mortality, clinical signs, body weight, and food consumption
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation across groups
	Metric 18:	Sampling Adequacy	High	× 1	1	8/sex/group
	Metric 19:	Blinding of Assessors	High	× 1	1	Each formulation will be labeled with the date of preparation, the study code number, a random five-digit concentration code number and a unique color code to insure that the study is conducted blind for dose. Animal cage cards will be similarly labeled with the study code number, concentration code number and color code. Personnel involved in animal dosing, animal care, and toxicologic and reproduction evaluations will not be informed of the formulation concentrations until such time as all laboratory .work has been completed.
	Metric 20:	Negative Control Response	High	× 1	1	Control data reported. No deviations from expected.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Body weight and food consumption data reported. Adult body weight changes <20%. No reported changes in food consumption.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No mortality or clinical signs.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	ANOVA and Duncan's Multiple Range test.
	Metric 24:	Reporting of Data	High	× 2	2	Quantitative Mortality, BW, and food consumption data. Qualitative reporting of lack of clinical signs.
Overall Quality Determination[‡]			High		1.0	
Extracted			Yes			

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Study Citation:	George, JD; Reel, , JR; Myers, CB; Lawton, AD; Lamb, JC (1986). Trichloroethylene: Reproduction and fertility assessment in F344 rats when administered in the feed NTP 86 312 PP
Data Type:	Continuous Breeding study - Task 1
HERO ID:	723905

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

8 Mechanistic

Table 246: **In vitro evaluation results of Cummings et al 2000 for in vitro cytotoxicity study on kidney outcomes**

Study Citation:	Cummings, BS; Lash, LH (2000). Metabolism and toxicity of trichloroethylene and S-(1,2-dichlorovinyl)-L-cysteine in freshly isolated human proximal tubular cells Toxicological Sciences, 53(2), 458-466					
Data Type:						
HERO ID:	194686					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name and molecular structure
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained commercially
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity reported as 99.9% assessed analytically
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control was used in all experiments
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical or necessary for cytotoxicity and metabolism kinetic assays
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described and appropriate for the endpoints
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Test results assessed by statistical comparison to control.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance storage was not described, but it is assumed that the test substance was stored according to manufacturer recommendation.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure details were reported clearly and there is no indication of inconsistent administration across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported clearly.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was clearly reported and sufficient to induce expected effects.
	Metric 12:	Exposure Route and Method	High	× 1	1	Number and spacing of concentrations were reported and appear appropriate for the assessment.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolically competent cells (freshly isolated primary human renal proximal tubule) were used, obviating the need for metabolic activation
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model was described and appropriate (freshly isolated primary human renal proximal tubule cells used to assess renal toxicity).

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Study Citation: Cummings, BS; Lash, LH (2000). Metabolism and toxicity of trichloroethylene and S-(1,2-dichlorovinyl)-L-cysteine in freshly isolated human proximal tubular cells Toxicological Sciences, 53(2), 458-466
Data Type:
HERO ID: 194686

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	Cells isolated from kidney slices from 2 or 3 patients used in each experiment.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Cytotoxicity in human cells assessed as decrease in total LDH activity because TRI inhibits LDH activity in human cells; this differs from rats (not tested in this study), and S-(1,2-dichlorovinyl)-L-cysteine experiments (this study), in which TRI cytotoxicity was measured as LDH release.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	There were no documented inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling appeared to be appropriate for the endpoints of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Only objective endpoints were assessed.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There was no indication in the study of differences among study groups that could have confounded the results.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Data analysis methods were appropriate for the data.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	No scoring or evaluation criteria have been established for these tests.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity was an evaluated endpoint, and methods to evaluate it were described.
	Metric 25:	Reporting of Data	High	× 2	2	Data presentation was adequate (presented graphically).
Overall Quality Determination‡			High		1.1	
Extracted			Yes			

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Study Citation:	Cummings, BS; Lash, LH (2000). Metabolism and toxicity of trichloroethylene and S-(1,2-dichlorovinyl)-L-cysteine in freshly isolated human proximal tubular cells Toxicological Sciences, 53(2), 458-466
Data Type:	
HERO ID:	194686

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 247: **In vitro evaluation results of Saillenfait et al 1995 for rat whole embryo culture on developmental toxicity outcomes**

Study Citation:	Saillenfait, AM; Langonne, I; Sabate, JP (1995). Developmental toxicity of trichloroethylene, tetrachloroethylene and four of their metabolites in rat whole embryo culture Archives of Toxicology, 70(2,2), 71-82
Data Type:	Embryonic toxicity in whole embryo culture
HERO ID:	630939

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name, and CASRN.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source was named.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity stated and analyzed by GC (99.5%).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Assay Procedures	Medium	× 1	2	Procedures were partially described and some assays were cited in another publication.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation was partially described and storage was not described.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The exposure doses/concentrations or amounts of test substance were reported without ambiguity
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration of 46 hours was reported.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups was reported, and dose/concentration spacing were not justified.
	Metric 13:	Metabolic Activation	High	× 1	1	Hepatic subcellular fractions were well described in terms of the experiments conducted.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was well-described and suitable for the outcomes of interest.
	Metric 15:	Number per Group	High	× 1	1	The number of organisms or tissues per study group and/or number of replicates per study group were reported and were appropriate for the study type and outcome analysis (n=12-20 embryos per group)
Domain 5: Outcome Assessment						

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Study Citation:	Saillenfait, AM; Langonne, I; Sabate, JP (1995). Developmental toxicity of trichloroethylene, tetrachloroethylene and four of their metabolites in rat whole embryo culture Archives of Toxicology, 70(2,2), 71-82
Data Type:	Embryonic toxicity in whole embryo culture
HERO ID:	630939

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Methods were not well-described in terms of specific details, but some methods were included in other publications.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Low	× 1	3	Blinding was not reported and outcome methodology for some evaluated endpoints was not sufficiently describe to determine if subjective.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences among the study groups,
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were described in limited details.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 248: **In vitro evaluation results of Drake et al 2006 for in vitro (embryonic) avian study on cellularity, cardiovascular development outcomes**

Study Citation:	Drake, V; Koprowski, S; Lough, J; Hu, N; Smith, S (2006). Trichloroethylene exposure during cardiac valvuloseptal morphogenesis alters cushion formation and cardiac hemodynamics in the avian embryo Environmental Health Perspectives, 114(6), 842-847
Data Type:	In vitro chick Embryo survival, valvuloseptal cellularity, and cardiac hemodynamics for TCE, TCOH, and TCA
HERO ID:	700370

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substances identified by name and (for TCE) molecular formula.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source was identified (Sigma-Aldrich)
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of test materials was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative vehicle (PBS) controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not applicable for the assays.
	Metric 6:	Assay Procedures	High	× 1	1	All assay details were described in details.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	QC criteria not applicable for the assays.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported but minor details (i.e., how pH was adjusted) were not reported. Solutions were prepared immediately prior to use.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported in both ppb and nmol
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration and frequency (four injections over specific developmental stages) were reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	There were three exposure groups with an overall range of 100x and the concentrations were chosen to bracket the EPA MCL.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation not required for the assays.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (white leghorn chicken eggs, Babcock and Bovan strains) was described, sources (Univ Wisconson-Madison and Utah State Univ) were reported, and model is sensitive for the outcome of interest.

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Study Citation:	Drake, V; Koprowski, S; Lough, J; Hu, N; Smith, S (2006). Trichloroethylene exposure during cardiac valvuloseptal morphogenesis alters cushion formation and cardiac hemodynamics in the avian embryo Environmental Health Perspectives, 114(6), 842-847
Data Type:	In vitro chick Embryo survival, valvuloseptal cellularity, and cardiac hemodynamics for TCE, TCOH, and TCA
HERO ID:	700370

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 15: Number per Group	High	× 1	1	The numbers of embryos per group were reported for each outcome and appeared appropriate.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodologies were fully described and sensitive.
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups (for example, cellularity counts made at same position in heart)
	Metric 18: Sampling Adequacy	High	× 2	2	Sampling for each outcome was reported and appeared to be adequate.
	Metric 19: Blinding of Assessors	High	× 1	1	Blinding was reported for apoptosis assessment; not applicable to other outcomes.
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences related to test design and procedures that influenced the outcome assessment.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences among the study replicates or groups in test model unrelated to exposure
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	High	× 1	1	Statistical methods were described and appropriate.
	Metric 23: Data Interpretation	Not Rated	NA	NA	The metric is not applicable.
	Metric 24: Cytotoxicity Data	Not Rated	NA	NA	This metric is not applicable.
	Metric 25: Reporting of Data	High	× 2	2	The data were reported for all outcomes and treatment groups (mean, SE, and n/group).
Overall Quality Determination‡		High		1.1	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 249: **In vitro evaluation results of Boyer et al 2000 for an in vitro cardiac development study**

Study Citation:	Boyer, A; Finch, W; Runyan, R (2000). Trichloroethylene inhibits development of embryonic heart valve precursors in vitro Toxicological Sciences, 53(1), 109-117
Data Type:	In vitro chick embryo cardiac development
HERO ID:	701307

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name as trichloroethylene.
	Metric 2:	Test Substance Source	Low	× 1	3	Source not identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative vehicle (medium) controls were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control were not required.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were partially described and cited to other publications but appeared to be appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Standards were not required.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Details regarding incorporation of TCE in the culture media and storage prior to use were omitted.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were reported or inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported (ppm).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was 48 hours.
	Metric 12:	Exposure Route and Method	High	× 1	1	There were five concentration groups and spacing rationale was reported for the measurement of epithelial-mesenchymal cell transformation assay. All other endpoints were measured in the control and high concentration groups only.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model was described and appropriate; source (Rosemary Farm) was reported.
	Metric 15:	Number per Group	High	× 1	1	The numbers of cells or explants per group were reported for each experiment/outcome and appeared to be appropriate.

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Study Citation:	Boyer, A; Finch, W; Runyan, R (2000). Trichloroethylene inhibits development of embryonic heart valve precursors in vitro Toxicological Sciences, 53(1), 109-117
Data Type:	In vitro chick embryo cardiac development
HERO ID:	701307

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodologies were described in detail and appeared appropriate.
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across treatment groups
	Metric 18: Sampling Adequacy	High	× 2	2	Sampling numbers were reported and appeared adequate.
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	Blinding not required.
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences among the study replicates or groups in test model unrelated to exposure and the test substance did not interfere with the assay
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	High	× 1	1	Statistical analysis was described (Student's t-test) and results reported. Data for each outcome were reported in tables or graphically allowing independent statistical analysis.
	Metric 23: Data Interpretation	Not Rated	NA	NA	Metric not applicable.
	Metric 24: Cytotoxicity Data	Not Rated	NA	NA	Metric not applicable.
	Metric 25: Reporting of Data	High	× 2	2	Data were reported for all outcomes in figures, tables, or text (including mean, SE, and n)
Overall Quality Determination‡		High		1.3	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 250: **In vitro evaluation results of Collier et al 2003 for a gene expression changes in embryonic cardiac cells after exposure study**

Study Citation:	Collier, JM; Selmin, O; Johnson, PD; Runyan, RB (2003). Trichloroethylene effects on gene expression during cardiac development Birth Defects Research, Part A: Clinical and Molecular Teratology, 67(7,7), 488-495
Data Type:	Gene expression changes in embryonic cardicac cells after exposure
HERO ID:	701547

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Test substance identified by name only but other publication was referenced.
	Metric 2:	Test Substance Source	Low	× 1	3	Source of test substance not reported, but other publication was referenced.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported but other publication was referenced.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls were included .
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were generally well described, however some details such as detection wavelenghts and concentrations were missing.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Limited details were provided but this is not expected to have a substantial impact.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups .
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and suitable for the outcomes of interest.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups and dose/concentration spacing were not explicitly justified by study authors but match doses uses in other TCE studies by this laboratory where developmental toxicity was observed.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was appropriate for the outcomes of interest, but limited details were provided. on rat source or husbandry.

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Study Citation:	Collier, JM; Selmin, O; Johnson, PD; Runyan, RB (2003). Trichloroethylene effects on gene expression during cardiac development Birth Defects Research, Part A: Clinical and Molecular Teratology, 67(7,7), 488-495
Data Type:	Gene expression changes in embryonic cardicac cells after exposure
HERO ID:	701547

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	Low	× 1	3	The number of rats per study group per study group were not reported but may be described partially in cited publication (Dawson et al, 1990). The number of replicates are also not reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcomes of interest .
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	Low	× 2	6	Details regarding sampling of outcomes were not fully reported , including number of replicates for both rats and genomic assays.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study groups
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	None were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	Data manipulations and calculations were described in limited details
	Metric 23:	Data Interpretation	Medium	× 2	4	Upregulation and downregulation of genes were reported but numerical assignment was not given. The descriptors strongly and greatly were used to describe up- and down-regulation, respectively, but no quantitative data was provided.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.
Overall Quality Determination[‡]			~~High~~ ⟶ Medium[§]		~~1.7~~	
Extracted			Yes			

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Study Citation:	Collier, JM; Selmin, O; Johnson, PD; Runyan, RB (2003). Trichloroethylene effects on gene expression during cardiac development Birth Defects Research, Part A: Clinical and Molecular Teratology, 67(7,7), 488-495
Data Type:	Gene expression changes in embryonic cardicac cells after exposure
HERO ID:	701547

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study
§ Evaluator's explanation for rating change: "Details are missing concerning technical and biological replicates and methods of administration."

Table 251: **In vitro evaluation results of Hassoun et al 2005 for a developmental toxicity to zebrafish embryos (dichloroacetate, metabolite exposure) study on developmental toxicity outcomes**

Study Citation:	Hassoun, E; Kariya, C; Williams, F (2005). Dichloroacetate-induced developmental toxicity and production of reactive oxygen species in zebrafish embryos Journal of Biochemical and Molecular Toxicology, 19(1), 52-58
Data Type:	Effects on zebrafish embryos for DCA
HERO ID:	702331

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as DCA (sodium dichloroacetate).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported. Although a batch/lot number was not reported, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The reported test substance purity (98%) is such that effects likely due to the test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using concurrent negative controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required by study type.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described adequately. Minor details were cited to other publications.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation (in water) was reported. Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported (i.e., from 4 to 144 hours post-fertilization).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups was reported (i.e., 4 groups plus controls). Although a rationale for dose selection was not specified, the doses used were adequate to show dose-response relationships.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required by study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was described and are commonly used for developmental outcomes. Parental fish were purchased from a pet store.

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Study Citation:	Hassoun, E; Kariya, C; Williams, F (2005). Dichloroacetate-induced developmental toxicity and production of reactive oxygen species in zebrafish embryos Journal of Biochemical and Molecular Toxicology, 19(1), 52-58
Data Type:	Effects on zebrafish embryos for DCA
HERO ID:	702331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of embryos was appropriate. The study indicated that tests were conducted in quadruplicate (5 embryos/experiment).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcomes methodology was described and addressed the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Low	× 1	3	Blinding was not reported; some developmental landmarks may have been subjective (e.g., behavioral effects).
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables were observed or reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables were observed or reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was described and appropriate.
	Metric 23:	Data Interpretation	Medium	× 2	4	Statistical significance was used as a criterion for a positive response. The dose-relatedness of the response was also presumably considered.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 252: **In vitro evaluation results of Hunter et al 1996 for a developmental-whole embryo (3-6 somites) culture exposed for 24 hours (TCE metabolites) study on developmental toxicity outcomes**

Study Citation:	Hunter, E; Rogers, E; Schmid, J; Richard, A (1996). Comparative effects of haloacetic acids in whole embryo culture Teratology, 54(2), 57-64
Data Type:	Whole embryo effects for TCA and DCA
HERO ID:	704439

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE metabolites were identified by name (tri- and dichloroacetic acid).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substances was reported. Although batch/lot numbers were not provided, the test substances are not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of the test substances were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using concurrent negative controls (conceptuses grown in control medium).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required by study type.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were adequately described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported (i.e., dissolved in deionized water). Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity (e.g., in Table 1).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (24 hours).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups was reported (6 or 7 groups plus controls). A rationale for dose selection was not provided.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 4: Test Model						

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Study Citation: Hunter, E; Rogers, E; Schmid, J; Richard, A (1996). Comparative effects of haloacetic acids in whole embryo culture Teratology, 54(2), 57-64
Data Type: Whole embryo effects for TCA and DCA
HERO ID: 704439

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	The test model was described and appropriate. CD-1 mice were obtained from a commercial source. It was stated that early stage somite staged conceptuses were obtained from females on gestation day 9; procedures for culturing were briefly described and cited to another publication.
	Metric 15:	Number per Group	Medium	× 1	2	The number of embryos per group was reported. Although there was variation in the number of embryos/group, numbers appeared to be sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was adequately described.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Low	× 1	3	Blinding was not reported but may have been warranted for determination of abnormalities (although accepted criteria were utilized, evaluation of some outcomes were presumably subjective).
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The study authors acknowledged potential changes in pH (based on increasing doses of TCA and/or DCA) and determined that these changed were unlikely to substantially impact the study results.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables in outcomes unrelated to exposure were observed. It is noted that TCA- and DCA-treated embryos experienced no mortality.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were described and were appropriate.
	Metric 23:	Data Interpretation	Medium	× 2	4	The criteria for a positive response were not explicitly specified; however, the statistical significance and dose-relatedness of the responses were presumably considered.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡			High		1.5	

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Study Citation:	Hunter, E; Rogers, E; Schmid, J; Richard, A (1996). Comparative effects of haloacetic acids in whole embryo culture Teratology, 54(2), 57-64
Data Type:	Whole embryo effects for TCA and DCA
HERO ID:	704439

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i \left(\text{Metric Score}_i \times \text{MWF}_i\right) / \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 253: **In vitro evaluation results of Loeber et al 1988 for a developmental cardiotoxicity in chick embryos study on cardiovascular development outcomes**

Study Citation:	Loeber, C; Hendrix, M; Diez De Pinos, S; Goldberg, S (1988). Trichloroethylene: A cardiac teratogen in developing chick embryos Pediatric Research, 24(6), 740-744					
Data Type:	Developmental cardiotoxicity in chick embryos					
HERO ID:	706804					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name, molecular weight and formula.
	Metric 2:	Test Substance Source	High	× 1	1	Source was identified.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity and grade were reported and such that effects likely due to test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A negative and vehicle concurrent control group was included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described in detail.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	No criteria were required for the test.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation and storage conditions were reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The embryo stage at injection and examination were reported, so an approximation of exposure could be calculated.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups and concentration spacing were reported and justified.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was described and appropriate.
	Metric 15:	Number per Group	High	× 1	1	The number of organisms was appropriate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was described.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate.

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Study Citation:	Loeber, C; Hendrix, M; Diez De Pinos, S; Goldberg, S (1988). Trichloroethylene: A cardiac teratogen in developing chick embryos Pediatric Research, 24(6), 740-744
Data Type:	Developmental cardiotoxicity in chick embryos
HERO ID:	706804

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 19:	Blinding of Assessors	High	× 1	1	Test solutions were coded and only decodes after lesions were identified.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported. The authors did note that more cardiac defects were observed in the second-to-highest concentration, but could not determine a cause.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	Statistical methods were used but not described; however, data provided to conduct and analysis.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Metric was not applicable.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Metric was not applicable.
	Metric 25:	Reporting of Data	High	× 2	2	The data were reported.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 254: **In vitro evaluation results of Ou et al 2003 for an alteration of HSP90 and endothelial cell proliferation (blood vessel development) study on cardiovascular development outcomes**

Study Citation:	Ou, J; Ou, Z; Mccarver, D; Hines, R; Oldham, K; Ackerman, A; Pritchard, K (2003). Trichloroethylene decreases heat shock protein 90 interactions with endothelial nitric oxide synthase: implications for endothelial cell proliferation Toxicological Sciences, 73(1), 90-97
Data Type:	Alteration of HSP90 and endothelial cell proliferation (blood vessel development)
HERO ID:	707319

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name
	Metric 2:	Test Substance Source	High	× 1	1	Source identified
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required.
	Metric 6:	Assay Procedures	High	× 1	1	Procedures were described in detail.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	No criteria were required for the assays.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test storage preparation methods were reported, but methods to prevent loss and storage conditions were not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure durations were reported.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups and concentration spacing were reported. Concentrations were not justified, but a dose response relationship was observed.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was described.
	Metric 15:	Number per Group	Medium	× 1	2	The number of exposed cells was not reported, but may be found in Arnal et al., 1994. Cells were used between passages 6 and 8.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was described.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required for the assays.

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Study Citation:	Ou, J; Ou, Z; Mccarver, D; Hines, R; Oldham, K; Ackerman, A; Pritchard, K (2003). Trichloroethylene decreases heat shock protein 90 interactions with endothelial nitric oxide synthase: implications for endothelial cell proliferation Toxicological Sciences, 73(1), 90-97
Data Type:	Alteration of HSP90 and endothelial cell proliferation (blood vessel development)
HERO ID:	707319

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	High	× 1	1	Statistical methods were appropriate.
	Metric 23: Data Interpretation	Not Rated	NA	NA	Interpretation criteria were not required.
	Metric 24: Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity analysis not required.
	Metric 25: Reporting of Data	High	× 2	2	All data were reported.
Overall Quality Determination‡		High		1.4	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 255: **In vitro evaluation results of Drake et al 2006 for avian developmental toxicity study on developmental-cardiac outcomes**

Study Citation:	Drake, VJ; Koprowski, SL; Hu, N; Smith, SM; Lough, J (2006). Cardiogenic effects of trichloroethylene and trichloroacetic acid following exposure during heart specification of avian development Toxicological Sciences, 94(1), 153-162
Data Type:	Avian heart development
HERO ID:	729401

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	Source and number were provided.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were included (vehicle controls).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required.
	Metric 6:	Assay Procedures	High	× 1	1	Assays procedures were well described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Criteria were not required.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported, but methods to prevent volatilization were not described. Storage following preparation was not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure durations were reported.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of groups was reported and the spacing was reported, and justified.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The model and source were described and commonly used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	The number of embryos evaluated per exposure was reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported and appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate.
	Metric 19:	Blinding of Assessors	High	× 1	1	Cell proliferation and death assays were blinded.

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Study Citation:	Drake, VJ; Koprowski, SL; Hu, N; Smith, SM; Lough, J (2006). Cardiogenic effects of trichloroethylene and trichloroacetic acid following exposure during heart specification of avian development Toxicological Sciences, 94(1), 153-162
Data Type:	Avian heart development
HERO ID:	729401

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	High	× 1	1	Statistical methods and data calculations were reported and appropriate.
	Metric 23: Data Interpretation	Not Rated	NA	NA	Evaluation criteria were not required.
	Metric 24: Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoints were described and appropriate.
	Metric 25: Reporting of Data	High	× 2	2	Data were reported.
Overall Quality Determination‡		High		1.3	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 256: **In vitro evaluation results of Caldwell et al 2008 for a cardiac gene expression and Ca in rat myocytes study on cardiovascular development outcomes**

Study Citation:	Caldwell, PT; Thorne, PA; Johnson, PD; Boitano, S; Runyan, RB; Selmin, O (2008). Trichloroethylene disrupts cardiac gene expression and calcium homeostasis in rat myocytes Toxicological Sciences, 104(1), 135-143
Data Type:	Cardiac gene expression and Ca in rat myocytes
HERO ID:	729622

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	Source identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls cultures were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Criteria were not required.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Preparation of the test substance was reported and steps were taken to minimalize volatization.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Durations were reported.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and concentration spacing were reported, and were justified.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model was identified and appropriate.
	Metric 15:	Number per Group	Medium	× 1	2	The number of cells per concentration were not reported, but cells were grown to 90% confluence.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcomes assessment methodology was reported and appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not required.
Domain 6: Confounding / Variable Control						

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Study Citation:	Caldwell, PT; Thorne, PA; Johnson, PD; Boitano, S; Runyan, RB; Selmin, O (2008). Trichloroethylene disrupts cardiac gene expression and calcium homeostasis in rat myocytes Toxicological Sciences, 104(1), 135-143
Data Type:	Cardiac gene expression and Ca in rat myocytes
HERO ID:	729622

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each treatment group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods and calculations were reported and appropriate.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Criteria were not required.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity measurements were not required.
	Metric 25:	Reporting of Data	High	× 2	2	All data were reported.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 257: **In vitro evaluation results of Rufer et al 2010 for a cardiac development in avian embryos study on cardiovascular development outcomes**

Study Citation:	Rufer, ES; Hacker, TA; Flentke, GR; Drake, VJ; Brody, MJ; Lough, J; Smith, SM (2010). Altered cardiac function and ventricular septal defect in avian embryos exposed to low-dose trichloroethylene Toxicological Sciences, 113(2), 444-452
Data Type:	Cardiac developmentn in avian embyros
HERO ID:	730034

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name.
	Metric 2:	Test Substance Source	Low	× 1	3	Source not identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods were cited in another publication (Drake et al., 2006).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Criteria were not required.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Preparation and storage were not reported but may be available in Drake et al. 2006b.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported consistently.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	A single dose was administered and then embryos were examined later.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and spacing were reported and justified.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model and described and appropriate.
	Metric 15:	Number per Group	High	× 1	1	The number of embryos evaluated was reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported and appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were administered consistently.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not required.
Domain 6: Confounding / Variable Control						

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Study Citation:	Rufer, ES; Hacker, TA; Flentke, GR; Drake, VJ; Brody, MJ; Lough, J; Smith, SM (2010). Altered cardiac function and ventricular septal defect in avian embryos exposed to low-dose trichloroethylene Toxicological Sciences, 113(2), 444-452
Data Type:	Cardiac developmentn in avian embyros
HERO ID:	730034

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were described and appropriate..
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Evaluation criteria were not required.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity tests were not required.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 258: **In vitro evaluation results of Selmin et al 2008 for a calcium signaling pathways in murine embryonal carcinoma cells study on cardiovascular development outcomes**

Study Citation:	Selmin, OI; Thorne, PA; Caldwell, PT; Taylor, MR (2008). Trichloroethylene and trichloroacetic acid regulate calcium signaling pathways in murine embryonal carcinoma cells p19 Cardiovascular Toxicology, 8(2), 47-56
Data Type:	Ca2+ signalling pathways in murine embryonal carcinoma cells
HERO ID:	730120

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	Source identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were reported.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Criteria were not required.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Preparation was reported Solutions were added fresh every 24h and the air space was flushed with nitrogen gas to control for volatilization.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The concentrations were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Durations of exposure were reported.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups and concentration spacing were reported but not justified.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Test model was reported with little descriptive information.
	Metric 15:	Number per Group	Medium	× 1	2	Number of cells used was not reported, but cells were grown to 75-80% confluence.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported and appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were administered consistently.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate..
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required.

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Study Citation:	Selmin, OI; Thorne, PA; Caldwell, PT; Taylor, MR (2008). Trichloroethylene and trichloroacetic acid regulate calcium signaling pathways in murine embryonal carcinoma cells p19 Cardiovascular Toxicology, 8(2), 47-56
Data Type:	Ca2+ signalling pathways in murine embryonal carcinoma cells
HERO ID:	730120

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not .
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	High	× 1	1	Statistical methods were reported and appropriate.
	Metric 23: Data Interpretation	Not Rated	NA	NA	No criteria were required.
	Metric 24: Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity was not required.
	Metric 25: Reporting of Data	High	× 2	2	Data were reported.
Overall Quality Determination‡		High		1.4	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 259: **In vitro evaluation results of Makwana et al 2010 for an avian heart developmental gene expression changes study on cardiovascular development outcomes**

Study Citation:	Makwana, O; King, NM; Ahles, L; Selmin, O; Granzier, HL; Runyan, RB (2010). Exposure to low-dose trichloroethylene alters shear stress gene expression and function in the developing chick heart Cardiovascular Toxicology, 10(2), 100-107
Data Type:	Avian heart developmental gene expression changes
HERO ID:	730161

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name and molecular formula.
	Metric 2:	Test Substance Source	High	× 1	1	Source and catalog number provided.
	Metric 3:	Test Substance Purity	High	× 1	1	Reported purity is such that effects likely due to test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were well described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Criteria were not required.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Test substance preparation and storage were not reported, but methods were as reported in Drake et al., 2006. (700370)
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Methods were as reported in Drake et al., 2006. (700370)
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	A single injection was used. Embryos were allowed to develop for 24 or 48 hr post-injection. The exposure duration appeared to be adequate to address the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups and spacing were reported and justified. Most experiments were conducted at a single low concentration after results of an assay at a low and high concentration.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model and stages of development was reported with no additional details.
	Metric 15:	Number per Group	High	× 1	1	The number was reported and adequate for the outcome of interest.
Domain 5: Outcome Assessment						

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Study Citation:	Makwana, O; King, NM; Ahles, L; Selmin, O; Granzier, HL; Runyan, RB (2010). Exposure to low-dose trichloroethylene alters shear stress gene expression and function in the developing chick heart Cardiovascular Toxicology, 10(2), 100-107
Data Type:	Avian heart developmental gene expression changes
HERO ID:	730161

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not required.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were reported or identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No outcomes unrelated to exposure were reported or identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was conducted but not described. Independent statistical analysis may be conducted based on estimation from graphs.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Evaluation criteria were not required.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity data were not required.
	Metric 25:	Reporting of Data	Medium	× 2	4	Most but not all outcomes were reported including results from a western blot.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 260: **In vitro evaluation results of Mishima et al 2006 for an avian heart developmental defects study on cardiovascular development outcomes**

Study Citation:	Mishima, N; Hoffman, S; Hill, EG; Krug, EL (2006). Chick embryos exposed to trichloroethylene in an ex ovo culture model show selective defects in early endocardial cushion tissue formation Birth Defects Research, Part A: Clinical and Molecular Teratology, 76(7), 517-527
Data Type:	Avian heart developmental defects
HERO ID:	730179

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	Source identified.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity such that effects likely due to test substance (99.5% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Criteria were not required.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage were reported and appropriate. An assay was conducted to determine test substance concentration under test conditions.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	A single exposure was used.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups and spacing were reported but not justified.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model was described and appropriate.
	Metric 15:	Number per Group	High	× 1	1	The number per group was reported and was sufficient.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate.

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Study Citation:	Mishima, N; Hoffman, S; Hill, EG; Krug, EL (2006). Chick embryos exposed to trichloroethylene in an ex ovo culture model show selective defects in early endocardial cushion tissue formation Birth Defects Research, Part A: Clinical and Molecular Teratology, 76(7), 517-527
Data Type:	Avian heart developmental defects
HERO ID:	730179

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	Blinding was not required.
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	High	× 1	1	Statistical analysis was described and appropriate.
	Metric 23: Data Interpretation	Not Rated	NA	NA	Criteria were not required.
	Metric 24: Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity was not assessed.
	Metric 25: Reporting of Data	High	× 2	2	Data were presented.
Overall Quality Determination‡		High		1.3	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 261: **In vitro evaluation results of Makwana et al 2013 for a mechanism-developmental study on growth (early life) and development outcomes**

Study Citation:	Makwana, O., Ahles, L., Lencinas, A., Selmin, O. I., Runyan, R. B. (2013). Low-dose trichloroethylene alters cytochrome P450-2C subfamily expression in the developing chick heart Cardiovascular Toxicology, 13(1), 77-84					
Data Type:	Mechanism-developmental					
HERO ID:	1293434					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name and chemical structure.
	Metric 2:	Test Substance Source	High	× 1	1	The source and catalog number were reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity such that effects likely due to test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric was not applicable.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described adequately.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	this metric is anot applicable.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Test substance preparation and storage were not reported, but more details regarding dosing of eggs were cited to other references.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The duration of exposure was reported and appeared to be appropriate.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups and spacing were reported but not justified. However, they appeared to be adequate to address the outcome of interest.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric was not applicable.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model, stage of development, and source were reported.
	Metric 15:	Number per Group	High	× 1	1	The number per group and replicated were described and sufficient for analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was described and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.

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Study Citation:	Makwana, O., Ahles, L., Lencinas, A., Selmin, O. I., Runyan, R. B. (2013). Low-dose trichloroethylene alters cytochrome P450-2C subfamily expression in the developing chick heart Cardiovascular Toxicology, 13(1), 77-84
Data Type:	Mechanism-developmental
HERO ID:	1293434

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric was not applicable.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables in test design were reported or identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding outcomes unrelated to exposure were reported or identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was reported and appropriate.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	this metric was not applicable.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	this metric was not applicable.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all outcomes.
Overall Quality Determination‡			High		1.1	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 262: **In vitro evaluation results of Seo et al 2012 for mechanistic-allergic response study**

Study Citation:	Seo, M., Kobayashi, R., Okamura, T., Ikeda, K., Satoh, M., Inagaki, N., Nagai, H., Nagase, H (2012). Enhancing effects of trichloroethylene and tetrachloroethylene on type I allergic responses in mice Journal of Toxicological Sciences, 37(2), 439-445
Data Type:	Mechanistic-allergic response
HERO ID:	2128339

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name as trichloroethylene
	Metric 2:	Test Substance Source	High	× 1	1	The source (Nacalai Tesque Co Ltd.) was identified.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was provided (98%).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative controls were used. Authors did not specify whether untreated or vehicle controls were used but noted that the solvent (DMSO) did not affect experiments.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described and applicable for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	No standards were required.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Preparation was reported, but no information on methods used to prevent volatilization during preparation was reported. Storage information was not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and consistent across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported in mg/L
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Duration of exposure (30 min) was reported.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (3 plus control) was reported and concentrations justified (values similar to Japanese standard for drinking water). Tested concentrations yielded a range of responses.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The source, cell type, and culturing methods were reported.
	Metric 15:	Number per Group	High	× 1	1	The number of cells used and number of experiments (3 replicates) were reported.

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Study Citation:	Seo, M., Kobayashi, R., Okamura, T., Ikeda, K., Satoh, M., Inagaki, N., Nagai, H., Nagase, H (2012). Enhancing effects of trichloroethylene and tetrachloroethylene on type I allergic responses in mice Journal of Toxicological Sciences, 37(2), 439-445
Data Type:	Mechanistic-allergic response
HERO ID:	2128339

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The method for determining histamine release was partially reported and cited to another publication.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to outcome
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required for outcomes.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were described and data fully reported graphically.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Criteria not required.
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported graphically for all treatment groups (mean, SE, and number replicates) for the outcome of interest.
Overall Quality Determination‡			Unacceptable⋆⋆		1.3	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 263: **In vitro evaluation results of Williams et al 2006 for a Zebrafish development with DCA study**

Study Citation:	Williams, FE; Sickelbaugh, TJ; Hassoun, E (2006). Modulation by ellagic acid of DCA-induced developmental toxicity in the zebrafish (Danio rerio) Journal of Biochemical and Molecular Toxicology, 20(4), 183-190					
Data Type:	Zebrafish development with DCA					
HERO ID:	2325731					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance, sodium dichloroacetate, was identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source was identified (Sigma Chemical)
	Metric 3:	Test Substance Purity	High	× 1	1	Purity was 98%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative untreated controls included. DCA administered in buffered water
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not required.
	Metric 6:	Assay Procedures	High	× 1	1	Procedures were described in detail and appeared appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	QC criteria not applicable for endpoint.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported (sodium DCA dissolved in water and pH adjusted to 7.5), but storage was not. Study duration was 6 days (144 hours) so lack of storage information is not expected to significantly impact results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity (mM).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported (140 hours) and appeared appropriate.
	Metric 12:	Exposure Route and Method	Low	× 1	3	A single exposure group (32 mM) was used. The dose was not justified.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Activation not required.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (Danio rerio) and source (Scientific Hatcheries) were described and appropriate.
	Metric 15:	Number per Group	High	× 1	1	The number of embryos (5/sample) and replicates (3 per treatment group and endpoint) were reported and appropriate
Domain 5: Outcome Assessment						

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Study Citation:	Williams, FE; Sickelbaugh, TJ; Hassoun, E (2006). Modulation by ellagic acid of DCA-induced developmental toxicity in the zebrafish (Danio rerio) Journal of Biochemical and Molecular Toxicology, 20(4), 183-190
Data Type:	Zebrafish development with DCA
HERO ID:	2325731

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodologies were partially reported and cited to other publications . Methods appeared to be appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate (5 embryos/sample for superoxide anion and nitric oxide assays).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were described and appropriate (2-way ANOVA and Scheffe's S method as post hoc test)
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Metric not applicable to study type
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Metric not applicable to study type
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and treatment groups.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 264: **In vitro evaluation results of Jiang et al 2015 for a developmental toxicity study on developmental-cardiac outcomes**

Study Citation:	Jiang, Y; Wang, D; Zhang, G; Wang, G; Tong, J; Chen, T (2015). Disruption of cardiogenesis in human embryonic stem cells exposed to trichloroethylene Environmental Toxicology, 31(11), 1372-1380
Data Type:	
HERO ID:	3036232

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Name and CASRN provided
	Metric 2:	Test Substance Source	High	× 1	1	Source provided but no additional information was given. TCE was obtained from a manufacturer, therefore not requiring analytical composition. Batch/lot number should not be necessary, as TCE is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity was provided and any effects are likely to be due to the test substance
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent control group included. Negative controls were included for all assays, however the details of the controls were not described in the methods section (e.g. were they vehicle controls, similar volume, etc).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not required for this assay
	Metric 6:	Assay Procedures	High	× 1	1	Procedures were described but some details were omitted (concurrent control details). Concerns about concurrent control details were addressed in Metric 4. Methods and procedures are otherwise well described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	No mention of steps taken during preparation and storage to prevent loss from volatility, questionable stability in culture media. The initial stock concentration was also not reported, as well as how long it was stored.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures administered consistently
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations reported in the study
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration reported and was appropriate
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure groups and concentration rationale were reported.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable for this study

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Study Citation: Jiang, Y; Wang, D; Zhang, G; Wang, G; Tong, J; Chen, T (2015). Disruption of cardiogenesis in human embryonic stem cells exposed to trichloroethylene Environmental Toxicology, 31(11), 1372-1380
Data Type:
HERO ID: 3036232

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Cell line was appropriate. Details were not provided on the cell line such as passage number, etc. These are unlikely to affect results.
	Metric 15:	Number per Group	Medium	× 1	2	Numbers of cells used were appropriate. This metric was very difficult to score, and the metric may need updating. The only option for insufficient reporting of number of cells or replicates is marking the entire study Unacceptable, which is inappropriate for potentially minor absences of reporting. Lack of reporting number of replicates should not be assumed to make the study unusable, depending on the assay. All experiments were performed in triplicate and results were statistically analyzed. Cell number was reported for most experiments but was missing from the immunohistochemistry assay. Cell number was not reported for RNA preparations, however the amount of RNA was reported. This reporting deficiency is not expected to impact results.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcomes for this study were appropriate
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	No deficiencies were observed in consistency of outcome assessment
	Metric 18:	Sampling Adequacy	Medium	× 2	4	Adequate sampling was conducted. As mentioned for metric 15, cell number was not provided for the immunohistochemistry assay. It is unknown whether the number of evaluated EBs/well (20) is sufficient or not for the particular assay of measuring contraction.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Subjective outcomes were not assessed
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables described or inferred
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variables described or inferred
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Student's T-test and ANOVA were used appropriately

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Study Citation:	Jiang, Y; Wang, D; Zhang, G; Wang, G; Tong, J; Chen, T (2015). Disruption of cardiogenesis in human embryonic stem cells exposed to trichloroethylene Environmental Toxicology, 31(11), 1372-1380
Data Type:	
HERO ID:	3036232

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	Not Rated	NA	NA	The criteria for this metric are not applicable to this type of test
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoint and methods were ell defined (MTT for cell viability)
	Metric 25:	Reporting of Data	High	× 2	2	All outcomes were presented for the exposed groups
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 265: **In vitro evaluation results of Wirbisky et al 2016 for a zebrafish study on developmental toxicity - cardiac outcomes**

Study Citation:	Wirbisky, SE; Damayanti, N; Mahapatra, CT; Sepulveda, MS; Irudayaraj, J; Freeman, JL (2016). Mitochondrial dysfunction, disruption of f-actin polymerization, and transcriptomic alterations in zebrafish larvae exposed to trichloroethylene Chemical Research in Toxicology, 29(2), 169-179
Data Type:	
HERO ID:	3222714

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Established nomenclature used
	Metric 2:	Test Substance Source	High	× 1	1	Source name reported. TCE obtained by a manufacturer with provided purity. Other information not required for this manufactured substance.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity such that effects likely due to test substance
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Vehicle control used for microarray. Vehicle controls were included for all assays. However, vehicle control only matched the ethanol concentration of the lowest TCE dose. Using the ethanol concentration of the highest TCE dose would have been ideal to rule out effects from the ethanol.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not required for the experiments
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for these experiments
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Unclear if proper steps were taken to control for volatilization prior to exposure. There was no mention of storage of the stock solution, however based on the experiments described it appears that TCE would have been incubated for the duration of the experiments in a sealed glass vial, so opening the vial to measure or replenish TCE would not have been appropriate.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Consistent administration was reported
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentration was reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate
	Metric 12:	Exposure Route and Method	High	× 1	1	Multiple doses were used and the spacing was appropriate
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation not required for these assays
Domain 4: Test Model						

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Study Citation: Wirbisky, SE; Damayanti, N; Mahapatra, CT; Sepulveda, MS; Irudayaraj, J; Freeman, JL (2016). Mitochondrial dysfunction, disruption of f-actin polymerization, and transcriptomic alterations in zebrafish larvae exposed to trichloroethylene Chemical Research in Toxicology, 29(2), 169-179

Data Type:

HERO ID: 3222714

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The test model was appropriate
	Metric 15:	Number per Group	Medium	× 1	2	N=9 is fairly low number per group, considering the ease of obtaining large numbers of embryos, and the use in statistical analysis. However, 50 embryos were pooled for the genomic analyses, so these were not an issue.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome methodology addressed the intended outcome
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent
	Metric 18:	Sampling Adequacy	Medium	× 2	4	Sampling of the biological replicates was adequate. In addition to the low number of embryos for the cell biology experiments, the authors did not report how many slices or images were taken/used for quantification. Downgraded to medium.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not applicable
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were reported
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variables were reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was appropriate
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Biological significance cut-off values was provided for microarray results, Scored as N/A, as no official guideline standard is applicable. For microarrays, typically a 2fold change is used, but this is not required.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	No cytotoxicity analysis required
	Metric 25:	Reporting of Data	High	× 2	2	All results were reported in the reference or supplemental report
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	Wirbisky, SE; Damayanti, N; Mahapatra, CT; Sepulveda, MS; Irudayaraj, J; Freeman, JL (2016). Mitochondrial dysfunction, disruption of f-actin polymerization, and transcriptomic alterations in zebrafish larvae exposed to trichloroethylene Chemical Research in Toxicology, 29(2), 169-179
Data Type:	
HERO ID:	3222714

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 266: **In vitro evaluation results of Harris et al 2018 for chick embryo study on cardiac gene expression and echocardiography outcomes**

Study Citation:	A. P. Harris, K. A. Ismail, M. Nunez, I. Martopullo, A. Lencinas, O. I. Selmin, R. B. Runyan (2018). Trichloroethylene perturbs HNF4a expression and activity in the developing chick heart Toxicology Letters, 285 113-120
Data Type:	Chick embryo study - cardiac gene expression and echocardiography
HERO ID:	4724313

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Definitive identification by chemical name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source (Sigma, Aldrich)
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade were not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A vehicle control was used (Tyrode's solution).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Benfluorex was used as a hepatocyte nuclear factor 4 alpha (HNF4a) agonist to help elucidate the role for this transcription factor in heart defects caused by TCE exposure; however, it was not used to test validity of study methods.
	Metric 6:	Assay Procedures	High	× 1	1	Test methods were described in detail and were appropriate (included gene expression findings and cardiac function testing).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	No QC criteria were reported for the test methods.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	TCE was diluted in 1xTyrode's solution prior to in ovo injection. TCE storage was not described, and the authors do not describe procedures for minimizing loss due to volatility.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Single in ovo injection at a consistent volume of 50 uL.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported in several units (ppm/ppb, uM and nmol per egg).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Injected at Hamilton and Hamburger (HH) stage 13; hearts were dissected for gene expression or echocardiograph analysis at HH17 (~24 hours).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	5 concentrations plus vehicle control for gene expression analysis (spacing was adequate). Only one concentration was used for echocardiograph analysis.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Cardiac CYPs are present in check embryos.
Domain 4: Test Model						

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Study Citation:	A. P. Harris, K. A. Ismail, M. Nunez, I. Martopullo, A. Lencinas, O. I. Selmin, R. B. Runyan (2018). Trichloroethylene perturbs HNF4a expression and activity in the developing chick heart Toxicology Letters, 285 113-120
Data Type:	Chick embryo study - cardiac gene expression and echocardiography
HERO ID:	4724313

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	Fertilized chick eggs were obtained from a commercial source and incubated to appropriate HH stages.
	Metric 15:	Number per Group	Medium	× 1	2	Hearts were for gene expression analysis (each pool was from 2 to 25 hearts; larger numbers were used for stages 15–18). 3-6 embryos/group were used for echocardiograph analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Mechanisms were investigated using gene expression methods; cardiac function was also analyzed by echocardiograph methods..
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across developmental stages.
	Metric 18:	Sampling Adequacy	High	× 2	2	Pooling was used to ensure adequate sampling for gene expression analyses. Gene expression analysis calculated mean levels from three independent pools of hearts at each stage, and echiocardiographic analysis also used the mean from three independent experiments.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were assessed.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding variables were identified
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	The statistical test is reported for some, but not all study results. Student t-test was used for gene transcription findings with more than one concentration group; however independent pairwise tests for a predetermined set of comparisons, especially on a nonmonotonic dose curve, can be acceptable. T-test was appropriate for fractional shortening results. Additionally, doses on each graph were accompanied by differing marks {|, --, .} that were not explained.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	There was no subjective scoring in this study
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	

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Study Citation:	A. P. Harris, K. A. Ismail, M. Nunez, I. Martopullo, A. Lencinas, O. I. Selmin, R. B. Runyan (2018). Trichloroethylene perturbs HNF4a expression and activity in the developing chick heart Toxicology Letters, 285 113-120
Data Type:	Chick embryo study - cardiac gene expression and echocardiography
HERO ID:	4724313

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	Medium	× 2	4	All assay data were reported graphically (mean +/- SEM). The text of the results section describes when results were statistically significant, and significance thresholds are indicated in the figure legends, however {*/**} indicating significance was not actually shown on the graphs, a presumed mistake.
Overall Quality Determination‡		~~High~~ ⟶ Medium§		~~1.4~~	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Some missing statistical significance indicators on graphs, use of Student's t-test not ideal for multiple doses,"