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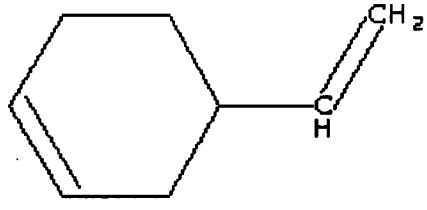
4-Vinylcyclohexene

Chemical Abstracts Service Registry Number: 100-40-3



U.S. EPA HPV Challenge Program Submission

Submitted by:

Synthetic Organic Chemical Manufacturers Association (SOCMA)
4-Vinylcyclohexene Work Group

Prepared by:

Experien Health Sciences, Inc.
6322 Water Point Court
Kingwood, Texas 77346
281-812-6667

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1. PLAIN LANGUAGE SUMMARY

Under the U.S. Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program, ExxonMobil Chemical Company and INVISTA S.à r.l committed thru the 4-Vinylcyclohexene Working Group of the Synthetic Organic Chemical Manufacturers Association (SOCMA) to voluntarily compile a Screening Information Data Set (SIDS) that can be used for an initial hazard assessment of 4-Vinylcyclohexene (4-VCH), CAS No. 100-40-3. Robust summaries have been prepared for all key studies. The information described in this test plan is a summary of the data presented in the Robust Summaries and should only be used for the purposes of HPV Program and not for regulatory cleanup or criteria development processes.

This test plan includes data for physicochemical, environmental fate, and mammalian and environmental effect endpoints included in the U.S. HPV Program in a manner consistent with the requirements of an OECD SIDS Level 1 data package. Additional mammalian data beyond the SIDS endpoints, and data / information on use and exposure, have also been supplied with this submission. Based on an exhaustive literature search, combined with data from accepted models to estimate partition coefficient, transport and distribution, photodegradation, and stability in water, adequate information is available for all endpoints.

4-VCH is commercially produced in closed continuous process systems via the catalytic dimerization of 1,3-butadiene. In addition, it is co-produced during the refining of crude butadiene and the production of dodecanedioic acid and vinylnorbornene. It is used as a chemical intermediate and is not known to be used directly as an ingredient in professional or consumer products (solvents, cleaners, adhesives, etc.). Given these conditions, exposures and releases to the environment are readily controlled and/or prevented.

4-VCH is not acutely toxic after inhalation, ingestion or skin contact, and no-more than moderately irritating to skin and eye. Results from repeated dose studies indicate that female mouse ovary is a potential target tissue, with alterations in other organs (including female rat ovary) expressed less consistently between species and sexes. Results from *in vitro* genetic toxicity testing have given mixed, predominately negative, findings while *in vivo* tests found no increase in micronuclei in rats and mice following high level, sub-chronic exposure. Interpretation of results from carcinogenicity data for 4-VCH in rats is confounded by poor survival; however the occurrence of ovarian tumors provided clear evidence of carcinogenicity in female mice. Ovarian toxicity was also apparent in a mouse continuous breeding study; however fertility and fetal development were unaffected. Structure-activity investigations indicate that metabolism of 4-VCH to a diepoxide is central to its ability to cause ovarian toxicity in the mouse.

If released to the environment, 4-VCH may pose moderate toxicity to aquatic and terrestrial organisms but it is not expected to bioaccumulate. Releases are predicted to partition primarily to air where it will undergo rapid photodegradation in the presence of atmospheric hydroxyl radicals and ozone. 4-VCH is not readily biodegradable by standard tests.

The table that follows summarizes the availability of data for each endpoint.

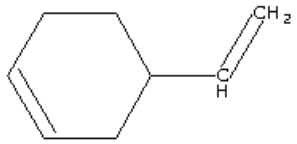
Data Availability Matrix

4-Vinylcyclohexene CASRN 100-40-3 HPV Endpoint	Measured Data Available?	Guideline Study?	GLP Study?	Supporting Information?	Estimation Method Used?	Data Acceptable?	Testing Recommended?
Physical / Chemical	Y = Yes, N = No						
Melting Point	Y	N	N	Y	N	Y	N
Boiling Point	Y	N	N	Y	N	Y	N
Density	Y	N	N	Y	N	Y	N
Vapor Pressure	Y	N	N	Y	N	Y	N
Partition Coefficient	N	N	N	N	Y	Y	N
Water Solubility	Y	N	N	Y	N	Y	N
Environmental Fate	Y = Yes, N = No						
Photodegradation	N	N	N	N	Y	Y	N
Stability in Water	N	N	N	N	Y	Y	N
Transport & Distribution	N	N	N	N	Y	Y	N
Biodegradation	Y	Y	Y	N	Y	Y	N
Bioaccumulation	Y	Y	Y	N	Y	Y	N
Ecotoxicity	Y = Yes, N = No						
Acute/Prolonged Fish	Y	Y	Y	Y	Y	Y	N
Acute Aquatic Invertebrates	Y	N	N	Y	Y	Y	N
Aquatic Plants	Y	N	N	Y	Y	Y	N
Chronic Fish	Y	N	N	N	Y	Y	N
Chronic Aquatic Invertebrates	Y	N	N	Y	Y	Y	N
Toxicity	Y = Yes, N = No						
Acute	Y	N	N	N	N	Y	N
Repeated Dose	Y	Y	Y	Y	N	Y	N
Genetic Toxicology in Vitro	Y	N	N	N	N	Y	N
Genetic Toxicology in Vivo	Y	N	Y	N	N	Y	N
Reproductive Toxicology	Y	N	N	Y	N	Y	N
Developmental Toxicology	N	N	N	Y	N	Y	N

Given the measured and estimated data available, the known hazards, and the circumstances under which this material is processed and used, no additional testing is being proposed.

2. CHEMICAL DESCRIPTION

4-Vinylcyclohexene (4-VCH, CASRN 100-40-3), a dimer of 1,3-butadiene, is a colorless liquid with the following chemical structure:



Molecular Formula: C8-H12
Molecular Weight: 108.18

4-VCH can be sold commercially at ≥ 97% pure. 4-VCH sold at high purity typically contains approximately 200 ppm of an appropriate oxidative inhibitor (e.g. *t*-butylcatechol). Impurities may include water and 1,5-Cyclooctadiene. Common synonyms for 4-Vinylcyclohexene include:

- 1,2,3,4-Tetrahydrostyrene
- 1-Cyclohexene, 4-vinyl-
- 1-Vinylcyclohexene-3
- 4-Ethenyl-1-cyclohexene
- 4-Ethenylcyclohexene
- 4-Vinylcyclohexene
- 4-Vinylcyclohexene-1

3. PRODUCTION, USE AND EXPOSURES

3.1. Production and Use

4-VCH production for commercial use occurs in closed continuous process systems via the catalytic dimerization of 1,3-butadiene. In addition, it is co-produced during the refining of crude butadiene and the production of dodecanedioic acid and vinylnorbornene. It is used as a chemical intermediate and is not known to be used directly as an ingredient of professional or consumer products.

3.2. Direct Worker Exposures

Because 4-VCH is produced and handled only in professional settings within closed systems, worker exposures are readily controlled and/or prevented. Workers can be exposed to fugitive emissions from process equipment during production and use and as well as during process sampling, filter changes, drumming activities, bulk loading activities, line clearing, and equipment maintenance and repair activities. Historical exposure monitoring data available in the literature (CMA, 1990; CMA, 1991) for on-purpose production of 4-VCH indicate that workplace breathing zone concentrations, as an 8-hour time-weighted average, are generally below the current TLV® of 0.1 ppm. There are no reliable estimates of the number of workers who might be exposed to 4-VCH during its production and use.

3.3. Indirect Worker Exposures

Workers can also be exposed to 4-VCH indirectly during the vulcanization of styrene-butadiene and polybutadiene rubber products, such as tires, shoe soles, hoses, power transmission belts, wire and cable products, and gaskets. In addition, workers may be exposed to 4-VCH as a result of passive emissions from styrene-butadiene (SB) latex adhesives used in the manufacture of carpets and laminated building materials. The 4-VCH is unintentionally formed in these products as a result of residual 1,3-butadiene monomer present. The nature and extent of exposures will depend largely on specific workplace conditions, but historical data available in the literature (Cocheo *et al*., 1983; Rappaport *et al*., 1977) suggests these exposures are below the current TLV® of 0.1 ppm. There are no reliable estimates of the number of workers who might be indirectly exposed to 4-VCH.

3.4. Indirect Consumer Exposures

Exposures to 4-VCH may also occur as a result of passive emissions from finished products such as carpets and laminated building materials where styrene-butadiene (SB) latex adhesives have been used during the manufacturing or installation process. With regards to carpets, residual monomer levels have trended downwards over the years and finished goods are increasingly being tested for conformance to various standards that limit total volatile organic emissions. These standards include the Carpet and Rug Institute "Green Label" and "Green Label Plus" testing programs, as well as various international standards. Environmental chamber studies suggest that airborne concentrations of 4-VCH from freshly milled and installed carpet will be in the order of a few parts per billion (ppb) and will decrease rapidly over several days as the carpet ages (Hodgson *et al*., 1993). Given these factors, indirect exposures to 4-VCH emissions from finished goods are expected to be negligible.

3.5. Releases to the Environment

Because 4-VCH is produced and handled only in professional settings within closed systems, environmental releases are readily controlled and/or prevented. There are no reliable estimates of the nature and extent of 4-VCH releases to the environment. However, in a survey conducted in response to EPA's 1991 Testing Consent Order for 4-VCH, manufacturers reported discharging 4-VCH to process sewers where it was sent to onsite wastewater treatment plants and destroyed before leaving the site (CMA, 1990). In a survey conducted prior to 1989 sponsored by the Effluent Guidelines Division of the U.S. EPA, 4-VCH was detected at waste water treatment facilities at 2 organics and plastics plants, 6 rubber processing plants, and 7 publicly owned treatment works at the following concentrations, respectively (USEPA, 1989):

- Median conc. 227 mg/L; max. conc. 446.7 mg/L
- Median conc. 78.8 mg/L; max. conc. 681.7 mg/L
- Median conc. 4.9 mg/L; max. conc. 8.5 mg/L

Releases to the atmosphere have not been reported in the literature but, given its volatility, low-level fugitive emissions can be expected.

4. PYSICOCHEMICAL PROPERTIES

The physicochemical properties of 4-VCH have been published in several references (handbooks) considered reliable for screening purposes. The data in the table below are considered definitive for each endpoint listed:

Property	Value	Rel †	Source
SIDS endpoints			
Melting point	-108.9°C	2	Lide, D.R. (ed.) (2004)
Boiling point	128.9°C	2	Lide, D.R. (ed.) (2004)
Relative density	0.8299 g/cm^3	2	Lide, D.R. (ed.) (2004)
Water solubility	50 mg/L @ 25°C	2	Yalkowsky, S.H.(2003)
Vapor pressure	15.7 mmHg @ 25˚C	2	Daubert, T.E. and Danner, R.P. (1994)
Log P_{ow}	3.93	2	MITI (1992)
Non-SIDS endpoints			
Flash point	15.85°C, open cup	2	Daubert, T.E. and Danner, R.P. (1994)
Autoflammability	269.85°C	2	Daubert, T.E. and Danner, R.P. (1994)

† Reliability according to Klimisch criteria

Conclusion: Adequate data are available to satisfy the required HPV physicochemical data elements for 4-VCH. No testing is proposed.

5. ENVIRONMENTAL FATE

5.1. Biodegradation

4-VCH is not expected to readily biodegrade. A MITI-1 ready biodegradability test was conducted on 4-VCH under aerobic conditions by following biochemical oxygen demand (BOD) in accordance with OECD 301C, with 0% degradation observed after 28 days (Chemicals Inspection & Testing Institute, 1992). The activated sludge concentration was 30 mg/L and the concentration of 4-VCH was 100 mg/L. Aniline was the reference substance used. Biodegradation was also determined using BCFWIN version 3.12. The program contains six models, three linear and three non-linear regressions. The rate of biodegradation, the time to primary and ultimate biodegradation, and whether the substance would pass the OECD 301C ready biodegradation test are determined. Ultimate biodegradation was predicted to take weeks.

Conclusion: Adequate data are available to satisfy this required HPV data element. No testing is proposed for this endpoint.

5.2. Photodegradation – Photolysis

No information on direct photolysis of 4-VCH was found. It is assumed to be insignificant compared to the reaction of 4-VCH to hydroxyl radicals and ozone in the atmosphere.

Conclusion: Experimental data on direct photolysis are not required under the HPV Program and, therefore, no testing is proposed.

5.3. Atmospheric Oxidation and Ozonation

With a vapor pressure of 15.7 mmHg at 25˚C, 4-VCH will volatilize to air where it is predicted to degrade rapidly through reactions with ozone (O_3) and photosensitized oxygen in the form of hydroxyl radicals (OH-). 4-VCH has been experimentally shown to react with ozone (Weschler, 1992). Using the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN, v1.91), 4-VCH has an estimated half-life, based on a 12-hour day, as follows:

Reaction	**Conc. of Sensitizer** (molecules/cm^3)	**Rate Constant** (cm3/molecule-sec)	**Est. Half-Life** (hours)	**Rel** †	**Source**
Ozone	7×10^{11}	21.2×10^{-17}	1.3	2	Modeled
OH-	1.5×10^{6}	89.3×10^{12}	1.4	2	Modeled

† Reliability according to Klimisch criteria

Conclusion: Adequate data on atmospheric oxidation and ozonation are available and, therefore, no testing is proposed.

5.4. Stability in Water – Hydrolysis

Stability in water has not been quantitatively evaluated for 4-VCH, because it does not contain functional groups susceptible to hydrolysis. The structure is that of an alicyclic hydrocarbon, a class of molecule not considered water reactive at relevant environmental pH values. Given these factors, hydrolysis is not expected to significantly contribute to the removal of 4-VCH from the environment. Furthermore, quantitative stability determinations (e.g. OECD 111) and modeling are considered unnecessary for compounds lacking hydrolysable functional groups.

Conclusion: Adequate technical understanding exists to satisfy this required HPV data element and, therefore, no testing is proposed.

5.5. Removal by Waste Treatment Plants

4-VCH will be readily removed from wastewater directed to sewage treatment plants with an estimated removal of at least 95% when modeled using the STPWIN™ subroutine in EPI Suite (v. 3.12). The model predicts that 78% of the 4-VCH will volatilize and that 15% will partition to sludge. Biodegradation accounted for <0.1% of total removal. Values were estimated using the following measured and calculated parameters: molecular weight, 108.18 g/mole; water solubility, 50 mg/L; vapor pressure, 15.77 mm Hg; Henry's Law constant, 0.0448 atm-m3/mole; octanol-water partition coefficient (Kow), 1.83; air-water partition coefficient (Kaw), 8511 (calculated by program); and log Kow, 3.93.

5.6. Distribution in the Environment (Fugacity Modeling)

Results of Mackay Fugacity Level I modeling indicate that environmental releases of 4-VCH will partition mainly to air while the Fugacity Model Level III program indicates that the majority will partition to the soil and water. These differing results can be explained by the model parameters, including the use of default emission rates and degradation half-lives. The Level I Fugacity model results are expected to provide a more representative prediction, based on the Henry's Law constant (HLC) of 0.0448 atm-m3/mole (HENRYWIN™ in EPI Suite v. 3.12) and organic carbon absorption coefficient (KOC) of 518 (Log Koc = 2.7) (PCKOCWIN™ in EPI Suite v. 3.12). Results of the two models are summarized in the table below:

Model Type	Compartment / Equilibrium Distribution (%)		Model Parameters	Model Source
Level I Fugacity	Air Water Soil Sediment	99.1 0.108 0.814 0.018	M.W.: 108.18 g/mole Temp.: 25°C Log Kow: 3.93 Water Solubility: 50 g/m3 Vapor Pressure: 2102 Pa Melting Point: -108.9°C	LEVEL 1 version 3.00 Fugacity-based model
Level III Fugacity	Air Water Soil Sediment	0.52 35.0 60.6 3.8	M.W.: 108.18 g/mole Temp.: 25°C Log Kow: 3.93 Water Solubility: 50 mg/L Vapor Pressure: 2102 Pa Soil Koc: 3.49×10^3	LEV3EPI™ Fugacity Model EPI Suite (v.3.12)

Conclusion: Adequate data are available to satisfy this required HPV data element. No testing is proposed for this endpoint.

5.7. Bioaccumulation Potential

4-VCH is not expected to bioaccumulate based on measured and estimated Bioconcentration Factors (BCF) as follows:

Species	Test Conc. (mg/L)	BCF	Rel †	Source
Carp (*Cyprinus carpio*)	10 100*	110 to 208 83 to 211	1 1	Chem. Insp. & Test Inst. (1992)
Calculated by Log Kow	Not applicable	212 (log BCF = 2.33)	2	Modeled BCFWIN v. 2.15

Model Parameters: Log Kow = 3.93 † Reliability according to Klimisch criteria *Saturated Solution

The carp noted above were exposed for 8 weeks under conditions according to the OECD 305C Bioconcentration Test as defined by the 12.05.1981 OECD Testing Guidelines for Chemicals. The carp were externally disinfected and sampled for mercury, acclimatized for 28 days, placed in 100 liter tanks under flow through conditions, and exposed to 4-VCH. The lipid content of the carp ranged from 2 to 6% with a mean of 4.1%. The two sets of BCF data indicate that 4-VCH has a low potential for bioaccumulation.

Conclusion: Adequate data are available to characterize the bioaccumulative potential of 4-VCH. No testing is proposed for this endpoint.

6. AQUATIC TOXICITY

4-VCH is expected to be moderately toxic to aquatic organisms, based on experimental data available for fish (*Oryzias latipes* or rice fish), invertebrate (*Daphnia magna*), and green alga (*Pseudokichneriella subcapitata*, former known as *Selenastrum capricornutum*). In addition, values have been estimated by structural activity relationships using Ecological Structural Activity Relationships (ECOSAR, v. 0.99h) for Microsoft Windows (10). The results of these studies and estimates are as follows:

Organism	Result (mg/L)	Rel †	Source
Acute Aquatic			
Orange-Red Killifish (*Oryzias latipes*) 96-hr LC_{50}	4.6	2	Ministry of Environment (2000)
Orange-Red Killifish (*Oryzias latipes*) 48-hr LC_{50}	17	2	Chem. Insp. & Test Inst. (1992)
Freshwater Fish Modeled 96-hr LC_{50}	1.23	2	Modeled (ECOSAR v. 0.99h)
Invertebrate (*Daphnia magna*) 48-hr EC_{50}	1.9	2	Ministry of Environment (2000)
	1.51	2	Modeled (ECOSAR v. 0.99h)
Green Alga (*Pseudokichneriella subcapitata*) 72-hr EC_{50}	>14	2	Ministry of Environment (2000)
Green Alga (*Pseudokichneriella subcapitata*) 48-hr EC_{50}	>14	2	Ministry of Environment (2000)
Green Alga (*Pseudokichneriella subcapitata*) 72-hr NOEC	7.7	2	Ministry of Environment (2000)
Green Alga (*Pseudokichneriella subcapitata*) 48-hr NOEC	>14	2	Ministry of Environment (2000)
Green Alga Modeled 96-hr EC_{50}	1.05	2	Modeled (ECOSAR v. 0.99h)
Chronic Aquatic			
Freshwater Fish Modeled 30-day ChV	0.22	2	Modeled (ECOSAR v. 0.99h)
Invertebrates (*Daphnia magna*) 21-day EC_{50}	0.92	2	Ministry of Environment (2000)
Invertebrates (*Daphnia magna*) 16-day EC_{50}	0.18	2	Modeled (ECOSAR v. 0.99h)
Invertebrates (*Daphnia magna*) 21-day NOEC	0.23	2	Ministry of Environment (2000)
Green Algae Modeled 96-h ChV	0.32	2	Modeled (ECOSAR v. 0.99h)
Terrestrial			
Earthworm Modeled 14-day LC_{50}	169 ppm*	2	Modeled (ECOSAR v. 0.99h)

Model Parameters: molecular weight = 108.18 g/mole; Log Kow = 3.93; Water Sol = 50 mg/L; Melting Pt. = -108.8°C; and SMILES Notation of C(=CCCC1C=C)C1. † Reliability according to Klimisch criteria *mg/kg soil

Conclusion: Adequate data are available to satisfy the required HPV data elements. No testing is proposed for this endpoint.

7. MAMMALIAN HEALTH EFFECTS DATA

Mammalian toxicity data for 4-VCH are summarized and discussed in the following sections. Additional data for studies beyond those required in the HPV Program are also presented.

7.1. Acute Toxicity

Adequate data are available for an assessment of the acute toxicity of 4-VCH in animals after inhalation, ingestion and skin contact and are summarized below. While no definitive value is available for lethality following short term inhalation exposure (with 4 of 6 rats dying after a 4 hr exposure to a limit dose of 8,000 ppm), it can be concluded that 4-VCH would not classified as highly toxic by inhalation. Data are also available on skin and eye irritation potential (non-SIDS endpoints).

Route	Species	Result	Comment	Rel †	Source
Inhalation LC_{50}	Rat	<8000 ppm	4-hr exposure	2	Smyth (1962); Smyth (1969)
Oral LD_{50}	Rat	2560 mg/kg bwt	gavage dosing	2	Smyth (1962); Smyth (1969)
Dermal LD_{50}	Rabbit	16600 mg/kg bwt ‡	24-hr occluded	2	Smyth (1962); Smyth (1969)
Irritation (non-SIDS)					
Skin Irritation	Rabbit	Moderate	24-hr occluded	2	Smyth (1962); Smyth (1969)
Eye Irritation	Rabbit	Slight	Undiluted	2	Smyth (1962); Smyth (1969)

† Reliability according to Klimisch criteria
‡ Reported as 20 ml/kg bwt; conversion based relative density = 0.8299 g/cm^3

Conclusion: Adequate data are available to satisfy the required HPV data elements. No testing is proposed for this endpoint.

7.2. Repeated Dose Toxicity

Results are available from a number of studies that have investigated the repeated dose toxicity of 4-VCH in rats or mice following exposure by inhalation or ingestion (oral gavage):

Species	Dose level	Duration	Source
Inhalation (ppm)			
Rat, Mouse	0, 240, 720, 1500	2 wk	Bevan *et al.* (1996)
Rat	0, 250, 1000, 1500	13 wk	
Mouse	0, 50, 250, 1000	13 wk	
Ingestion (mg/kg body weight/d)			
Rat, Mouse	0, 300, 600, 1250, 2500, 5000	2 wk	NTP (1986)
Rat	0, 50, 100, 200, 400, 800	13 wk	
Mouse	0, 75, 150, 300, 600 or 1200	13 wk	
Rat, Mouse	0, 200, 400	2 yr	

Findings from the sub-chronic (13 wk) and chronic (2 yr) investigations provide adequate screening level information on the hazards of repeated inhalation or ingestion (gavage) exposure to 4-VCH. These key studies, each with a high degree of reliability ($\geq$2) according to Klimisch criteria, are described in the paragraphs below and detailed further in the robust summaries. Results from the 2 week investigations are also summarized as robust summaries; however, since they were designed primarily for dose-range setting and contain little additional toxicological information, they will not be discussed further in this document.

Bevan *et al*. (1996) exposed groups of 10 male and female Sprague-Dawley rats or B6C3F1 mice to 4-VCH by inhalation 6 hours/day, 5 days/week for 13 weeks. All high-dose male and 8 of 10 female mice died prior to completion of the study, with most animals dying on or before day 12. For rats, a statistically significant incidence of lethargy was apparent in males at 250 ppm and in

both sexes at 1500 ppm. Reduced body weight and/or weight gain were observed for male and female rats exposed at 1000 ppm and 1500 ppm. Liver weights were significantly increased in male and female rats exposed ≥1000 ppm, and kidney weights in males exposed to ≥1000 ppm and females at 1500 ppm 4-VCH, however no histopathological anomalies were present. For mice, increased incidences of lethargy, mortality and ovarian atrophy (diagnosed by microscopic examination) were observed at 1000 ppm. Hematological, clinical chemistry and urinalysis parameters were unaffected by treatment in both species. These findings are consistent with a sub-chronic NOAEC of 250 ppm for 4-VCH in rats and mice.

In a 13 week sub-chronic gavage study reported by NTP (1986), male and female F344 rats and B6C3F1 mice were administered 4-VCH in corn oil, 5 days/week for 13 weeks. Findings in rats were limited to decreased body weight gain in males at ≥ 400 mg/kg body weight/day and females at 800 mg/kg/day; minimally increased severity of hyaline droplet degeneration of the renal proximal convoluted tubule of high dose males; and the occurrence of occasional inflammatory changes in non-glandular stomach from high dose males and females. In mice, a high level of early mortality was apparent in high dose animals of both sexes, although the toxicological relevance of this finding appears doubtful due to evidence of mis-dosing diagnosed at gross necropsy. Mild acute inflammation of the stomach was detected occasionally following microscopic examination of tissue from high dose males and females. Histological re-evaluation of ovaries from high dose females (subsequent to completion of the two year mouse study) revealed a reduction in the number of primary follicles and mature graafian follicles (lower dose groups not examined). No other microscopic tissue changes were present in mice. These findings point to a sub-chronic oral NOAEL of 200-400 mg/kg body weight/day for male and female rats, respectively, based on reduced body weight gain, and a marginal NOAEL of 600 mg/kg body weight/day for mice, reflecting occasional mild acute gastric inflammation detected in high dose animals. The occurrence of histopathological changes in mouse ovary is consistent with results obtained from other studies; however no no-effect level is available in this instance due to an absence of data for the lower treatment groups.

In a chronic gavage investigation (NTP, 1986), male and female F344 rats and B6C3F1 mice were administered 4-VCH in corn oil for 103 weeks. For rats, survival was significantly decreased by week 103 in males at all doses and in high-dose females. Both sexes also exhibited an increased incidence of epithelial hyperplasia of the forestomach (more pronounced in males), which was statistically significant in males surviving beyond week 93. For mice, survival was decreased in the high-dose animals of both sexes, with stomach abnormalities (including ulcers, inflammation, and epithelial hyperplasia of the forestomach) and lung congestion detected in survivors at necropsy. Histopathological examination revealed a significant increase in the incidence of hepatic centrilobular congestion and atrophy of spleenic red pulp in high dose males only, with adrenal gland congestion and cortex alterations and ovarian changes in females from both treatment groups. The microscopic changes present in ovary, which included tubular cell-, granulose cell-, and papillary-hyperplasia, appear biologically significant given the tumor and reproductive findings reported in other studies in mice (discussed further in sections 7.4 and 7.5, below). A chronic LOAEL of 200 mg/kg body weight per day is obtained from these studies based on decreased survival in male rats, and the occurrence of histological abnormalities in the stomach of rats and mice (both sexes), liver and spleen of male mice, and adrenal gland and ovary of female mice.

Overall, results from sub-chronic and chronic testing indicate that female mouse ovary is a potential target for 4-VCH-induced systemic toxicity, with changes in stomach in rats and mice detected following oral (gavage) administration. As indicated in the table below, alterations in other organs are expressed less consistently between species and sexes.

Species	Liver	Kidney	Ovary	Stomach	Adrenal	Spleen	Lung	NOAEC/L	Source
Inhalation (13-Week Study)									
Rat	M,F	M, F	---	---	---	---	---	250 ppm	Bevan *et al.* (1996)
Mouse	---	---	F	---	---	---	---	250 ppm	Bevan *et al.* (1996)
Ingestion (gavage, 13-Week Study)									
Rat	---	M	---	M, F	---	---	---	200-400 mg/kg/d	NTP (1986)
Mouse	---	---	F	M, F	---	---	---	600 mg/kg/d	NTP (1986)
Ingestion (gavage, 103-Week Study)									
Rat	---	---	---	M, F	---	---	---	<200 mg/kg/d	NTP (1986)
Mouse	M	---	F	M, F	F	M	M, F	<200 mg/kg/d	NTP (1986)

Conclusion: Adequate data are available to satisfy the required HPV data elements. No testing is proposed for this endpoint.

7.3. Genetic Toxicity

Adequate *in vitro* and *in vivo* data are available to characterize the genotoxicity of 4-VCH and its primary metabolites. A summary of the available information is presented below:

End point	Test system	Conditions	Result	Rel[†]	Source
In Vitro					
Gene Mutation	Bacterial Cells	S. typhimurium TA97, 98, 100, 104, 1535; liquid preincubation; hamster S9	Negative	2	NTP (1989)
		S. typhimurium TA98, 100, 1535, 1537; liquid preincubation; hamster S9	Negative	2	NTP (1981)
	Mammalian Cells	Mouse lymphoma cells (L5178Y TK+/-); rat S9	Positive	2	NTP (undated)
Sister Chromatid exchange	Mammalian Cells	Chinese Hamster Ovary (CHO)	Negative	2	NTP (1984)
Chromosomal Aberrations	Mammalian Cells	Chinese Hamster Ovary (CHO)	Negative	2	NTP (1984)
In Vivo					
Micronuclei	Bone marrow; SD rats	Inhalation; 0, 250, 1000, or 1500 ppm 4-VCH, 6 hr/day, 5 day/week, 13 weeks.	Negative	2	DuPont (1994)

Micronuclei	Bone marrow; B6C3F1mice	Inhalation; 0, 50, 250, or 1000 ppm 4-VCH, 6 hr/day, 5 day/week, 13 weeks.	Negative	2	DuPont (1994)
Metabolites (Summary Only)					
4-Vinylcyclohexene diepoxide induced gene mutation, sister chromatid exchange and chromosomal aberrations but not micronuclei in mammalian cells in vitro. It was mutagenic in bacteria and caused gene conversion and mitotic crossing-over in yeast cells (*Saccharomyces cerevisiae*). A metabolite of 4-vinylcyclohexene diepoxide, 4-epoxyethylcyclohexane-1,2-diol, was not mutagenic to *Salmonella typhimurium*. Two mono-epoxide metabolites, 4- Epoxyethylcyclohexene and 4-Vinyl-1,2-epoxycyclohexane, were not mutagenic to *Salmonella typhimurium*, but the latter induced micronuclei, but not hprt locus mutations, in cultured Chinese hamster cells.				2	IARC (1994)

† Reliability according to Klimisch criteria

Conclusion: Adequate data are available to satisfy the required HPV data elements. No testing is proposed for this endpoint.

7.4. Carcinogenicity (non-SIDS Endpoint)

NTP (1986) exposed male and female F344 rats and B6C3F1 mice to 4-VCH in corn oil by oral gavage at doses of 0, 200, or 400 mg/kg body-weight per day, 5 days per week, for 103 weeks. For rats, exposure to 4-VCH was associated with the occurrence of neoplastic lesions in skin, urinary bladder, pituitary, preputial gland and clitoral gland. For mice, exposure to 4-VCH was associated with the occurrence of neoplastic lesions in ovary, lung, hematopoietic system and adrenal gland. Unambiguous interpretation of these findings was confounded, however, by poor health and low survival which may have resulted in artefactual temporal and statistical associations between treatment and tumor incidence in animals dying from unrelated / undefined causes. Overall, NTP concluded that the study was inadequate and the results inconclusive with regard to the potential carcinogenicity of 4-vinylcyclohexene in the rat, but that the occurrence of ovarian tumors provided clear evidence of carcinogenicity of 4-vinylcyclohexene in the mouse.

Van Duureen et. al. (1963) exposed 30 male Swiss mice to a 50% solution of 4-VCH in benzene, applied to clipped dorsal skin. The solution was applied 3 times per week for approximately 54 weeks. Under the conditions of this study, dermal exposure to 4-VCH resulted in an increased number of benign squamous cell papillomas in male Swiss mice. One malignant tumor was also observed in the group treated with 4-VCH, but was considered by the authors to have resulted from spontaneus formation of 4-VCH hydroperoxide following autoxidation of the parent substance.

Conclusion: Carcinogenicity is not a required HPV data element. No testing is proposed.

7.5. Reproductive and Developmental Toxicity

7.5.1 Reproduction and fertility

Results are available from a continuous breeding study (Grizzle *et al*., 1994) in which F_0 male and female CD-1 mice were administered 4-VCH by oral gavage at doses of 0, 100, 250 or 500

mg/kg body weight/day for 16 weeks prior to conception of an F_1 breeding generation. Subsequently, direct dosing (0 or 500 mg/kg body weight/day, by gavage) of 21-day old weaning F_1 adults commenced 7-8 weeks prior to conception of an F_2 generation. As a result of the schedule adopted, adults were exposed to 4-VCH before and during mating and throughout pregnancy and lactation, with continuous exposure of the fetuses and pups occurring secondary to maternal treatment (i.e. occurring *in utero* or via milk, respectively). 4-VCH, at doses up to 500 mg/kg body weight/day, was without effect on reproductive performance of the F_0 or F_1 generations, including mating and fertility indices, live litter size, sex ratio and pup survival to post-natal day 4. Clear ovarian toxicity was apparent in F_1 females however, as evidenced by significant, marked (up to 50%) decrements in numbers of primordial oocytes, growing follicles and antral follicles together with slight (~15%), statistically significant reductions in sperm motility in F_1 males (concentration and morphology unaffected). These findings indicate that while 4-VCH is a gonadal toxicant in mouse ovary it did not adversely impact reproductive performance in F_0 or F_1 generations.

Mechanistic investigations have shown that female B6C3F1 mice are more sensitive to 4-VCH induced ovarian toxicity than female F344 rats (Smith *et al*., 1990a), with ED_{50} values (i.e. dose causing 50% reduction in oocyte numbers) of 2.7 and >7.4 mmol/kg body weight/day i.p., respectively. Oocytes from both species were sensitive to *in vivo* administration of the epoxide- and diepoxide metabolites of 4-VCH (ED_{50} values in range 0.2-1.4 mmol/kg/day), with ovarian toxicity in mice given 4-VCH reduced following inhibition of epoxide hydrolase activity (Smith *et al*., 1990a). Structure-activity investigations indicate that metabolism to a diepoxide is central to the induction of ovarian toxicity by 4-VCH in the mouse (Doerr *et al*., 1995), effects that occur without any alteration in plasma follicle stimulating hormone levels (Hooser *et al*., 1993).

7.5.2 Fetal development

In the mouse continuous breeding study described above (Grizzle *et al*., 1994), no adverse effects were reported on pregnancy or pre- and post-natal fetal development following exposure of two generations of pregnant female B6C3F1 mice to 4-VCH by gavage, at doses up to 500 mg/kg body weight/day. The results provide screening level information that 4-VCH is not fetotoxic or teratogenic in the mouse.

Conclusion: Adequate data are available to satisfy the required HPV data elements. No testing is proposed for this endpoint.

7.6. Metabolism and Toxicokinetics (non-SIDS Endpoint)

Information is available on the toxicokinetics of 4-VCH and its metabolites in mice and rats *in vivo* and *in vitro*, and on the transformation of 4-VCH by human liver preparations *in vitro*.

Urine and exhaled air are the main routes of excretion of 4-VCH-derived radioactivity following oral (gavage) administration to female rats and mice, with generally low levels of retention in both species (Smith *et al*., 1990b).

Mice metabolize 4-VCH to the 1,2 epoxide *in vivo* more readily than the rat (Smith *et al*., 1990b). Enzyme and antibody inhibition/induction studies demonstrate that constitutively-expressed hepatic microsomal cytochrome P450IIA and P450IIB are primarily responsible for this activity in female B6C3F1 mice, while cytochrome P450IIB present in female F344 rat liver is also able to perform this function but to a more limited extent (Smith *et al*., 1990c). Epoxide hydrolase is also involved in the disposition of 4-VCH (Smith *et al*., 1990d; Watabe *et al*., 1981), with rapid conversion of the 1,2- and 7,8 monoepoxides to the diol in both species. 4-VCH and its mono- or diepoxide metabolites rapidly decrease hepatic glutathione *in vivo*, while the diepoxide is a good substrate for mouse hepatic glutathione transferease (Giannarini *et al*., 1981).

Enzyme kinetic data demonstrate that processes leading to formation of 4-VCH epoxides and diepoxides *in vitro* are generally more active (higher V_{max}, lower K_m) in microsomal fractions from mouse liver and lung than in comparable tissue from rats. Hydrolysis of 4-VCH diepoxide was recorded in rat and mouse liver and lung and rat ovary (insufficient material for studies on mouse ovary), with the greatest V_{max} returned by rat liver (Keller *et al*., 1997).

Air:tissue partition coefficient data for 4-VCH and its 1,2- and 7,8-epoxides demonstrate a generally higher affinity for mouse tissues and blood than for the corresponding rat samples, with the exception of ovary (where values were generally greater for the rat) (Keller, 1993). The epoxides were consistently more soluble than the parent substance, with adipose tissue exhibiting the greatest affinity (Keller *et al*., 1993).

Human hepatic microsomal fractions metabolized 4-VCH to the 1,2- and 7,8-epoxides in vitro, with production of the 1,2-epoxide predominating (in a range 0.23 to 1.25 nmol/mg microsomal protein/min; formation of the 7,8-epoxide formation was around 6 fold slower) (Smith *et al*., 1991). This contrasts with rates of 4-VCH 1,2-epoxide formation by mouse hepatic microsomal fractions of 8-9 nmol/min/mg microsomal protein (Smith *et al*., 1990 b,d).

Species and tissue differences in activation and detoxication, as well as differences in tissue affinity and distribution, appear relevant to differences in susceptibility of rats and mice to 4-VCH-induced ovarian toxicity and neoplasia.

Conclusion: Metabolism and toxicokinetics are not a required HPV data element. No testing is proposed.

8. DATA AVAILABILITY AND TESTING PROPOSAL

Adequate physicochemical, environmental fate, aquatic toxicity, and mammalian toxicity data are available to address SIDS endpoints for 4-VCH. No further testing is proposed.

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