4-Vinylcyclohexene Group
1850 M Street, NW
Suite 700
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October 30, 2006November 13, 2006

Administrator
US Environmental Protection Agency
P. O. Box 1473
Merrifield, VA 22116
Attention: Chemical Right-to-Know Program

To Whom It May Concern:

The 4-Vinylcyclohexene Group comprised of ExxonMobil and INVISTA is providing the Agency with a robust summary and test plan for the chemical *Cyclohexene, 4-ethenyl-* (CAS RN 100-40-3), commonly known as 4-Vinylcyclohexene, or 4-VCH, under the auspices of the HPV Challenge Program. Enclosed is a computer disc containing the robust summary and test plan.

If you have any questions or need additional information, please contact me at (202) 721-4100.

Sincerely,

Richard E. Opatick
4-VCH Group, Director

cc: 4-VCH Membership

Enclosure – CD of Robust Summary and Test Plan

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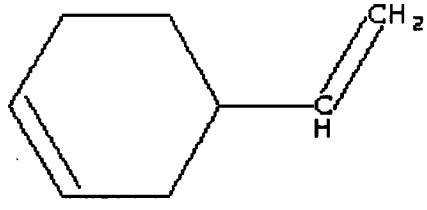
4-Vinylcyclohexene

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:

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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.

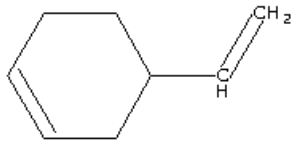
Data Availability Matrix

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.

References

Bevan C, Stadler JC, Elliott GS, Frame SR, Baldwin JK, Heung H-W, Moran E and Panepinto AS (1996) Subchronic toxicity of 4-vinylcyclohexene in rats and mice by inhalation exposure. Fund Appl Toxicol 32, 1-10.

CMA (Chemical Manufacturers Association) (1990) Report on the survey of the Butadiene Panel of the Chemical Manufacturers Association on 4-vinylcyclohexene. Submitted to the U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, D.C. May 3, 1990.

CMA (Chemical Manufacturers Association) (1991) Industrial hygiene sampling for 4-vinylcyclohexene in the workplace - final report. Submitted to the U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, D.C. U.S. EPA/OPTS Public Files, Fiche #: OTS0533179. Doc#:40-91109046. October 1, 1991.

Chemicals Inspection & Testing Institute (1992) Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. Ministry of International Trade and Industry, Published by Japan Chemical Industry Ecology-Toxicology & Information Center, Tokyo, Japan. ISBN: 4890741011.

Cocheo, V and Bellomo, ML (1983) Rubber manufacture: sampling and identification of volatile pollutants. Am Ind Hyg Assoc J 44, 521-527.

Daubert, T.E. and Danner, R.P. (1994) Physical and Thermodynamic Properties of Pure Chemicals Part 5. Taylor and Francis. p. not available.

Doerr, JK, Hooser, SB, Smith, BJ and Sipes, IG (1995) Ovarian toxicity of 4-vinylcyclohexene and related olefins in B6C3F1 mice: role of diepoxides. Chem Res Toxicol 8, 963-969.

DuPont (1994). Rat and Mouse Bone Marrow Micronucleus Assay of 4-Vinylcyclohexene Following Subchronic Inhalation Exposure Revision No. 1. Haskell Laboratory Report No. 506-93. February 22, 1994. EPA Docket #OPTS-42116.

Giannarini, C, Citti, L, Gervasi, PG and Turchi, G (1981) Effects of 4-vinylcyclohexene and its main oxirane metabolite on mouse hepatic microsomal enzymes and glutathione levels. Toxicol Lett 8, 115-121.

Grizzle, TB, George, JD, Fail, PA, et. al. (1994) Reproductive effects of 4-vinylcyclohexene in Swiss Mice assessed by a continuous breeding protocol. Fundam Appl Toxicol 22, 122-129.

Hodgson AT, Wooley JD, and Daisey, JM (1993) Emissions of volatile organic compounds from new carpets measured in a large-scale environmental chamber. Journal of the Air and Waste Management Association, 43, 316-324.

Hooser, SB, Parola, LR, Van Ert, MD and Sipes IG (1993) Short Communication: Differential ovotoxicity of 4-vinylcyclohexene and its analog, 4-phenylcyclohexene. Toxicol Appl Pharm 119, 302-305.

IARC (1994). International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans: 4-vinylcyclohexene. Vol 60, pages 347; 354-355; 361.

Keller, DA (1993) Partition coefficients of 4-vinyl cyclohexene and metabolites. Unpublished report, Haskell Laboratory Report No. 102-93 for Chemical Manufacturers Association, Washington DC, March 1993.

Keller, DA, Carpenter, SC, Cagen, SZ and Reitman, FA (1997) In vitro metabolism of 4-vinylcyclohexene in rat and mouse liver, lung and ovary. Toxicol Appl Pharmacol 144, 36-44.

Lide, D.R. (ed.) (2004) CRC Handbook of Chemistry and Physics. 85th Edition. CRC Press LLC Boca Raton, Florida p. 3-568.

Ministry of Environment (2000) 4-Vinylcyclohexene Ecotox data cited in the National Institute of Technology and Evaluation online database, Tokyo, Japan.

MITI (Ministry of International Trade and Industry) (1992). Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan.CITI ed. (Chemicals Inspection & Testing Institute). Published by Japan Chemical Industry Ecology-Toxicology & Information Center.

NTP (1981) National Toxicology Program Database Search Application. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm. CAS#100-40-3. Genetic Toxicity Studies. Salmonella. Study No. 777152. Detailed Study Data.

NTP (1984) National Toxicology Program Database Search Application. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm. CAS#100-40-3. Genetic Toxicity Studies. CHO Cell Cytogenetics-Chromosome Abberations. Study No. 169960. Detailed Study Data.

NTP (1984) National Toxicology Program Database Search Application. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm. CAS#100-40-3. Genetic Toxicity Studies. CHO Cell Cytogenetics-Sister Chromatid Exchange. Study No. 169960. Detailed Study Data.

NTP (1986) Toxicology and carcinogenesis studies of 4-vinylcyclohexene (CAS No. 100-40-3) in F344/N rats and B6C3F1 mice (gavage studies). NTP Technical Report 303, NIH Publication No. 86-2559, August 1986.

NTP (1989) National Toxicology Program Database Search Application. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm. CAS#100-40-3. Genetic Toxicity Studies. Salmonella. Study No. 609542. Detailed Study Data.

NTP (undated) National Toxicology Program Database Search Application. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm. CAS#100-40-3. Genetic Toxicity Studies. Mouse Lymphoma Studies. Study No. 971117. Detailed Study Data.

Rappaport, SM and Fraser, DA (1977) Air sampling and analysis in a rubber vulcanization area. Am Ind Hyg Assoc J, 38, 205-210.

Smith, BJ, Mattison, DR and Sipes, GI (1990a) The role of epoxidation in 4-vinyl cyclohexene-induce ovarian toxicity. Toxicol Appl Pharmacol 105, 371-381.

Smith, BJ, Carter, DE and Sipes, GI (1990b) Comparison of the disposition and in vitro metabolism of 4-vinyl cyclohexene in the female mouse and rat. Toxicol Appl Pharmacol 105, 364-371.

Smith, BJ, Sipes, IG, Stevens, JC and Halpert, JR (1990c) The biochemical basis for the species difference in hepatic microsomal 4-vinylcyclohexene epoxidation between female mice and rats. Carcinogenesis 11, 1951-1957.

Smith, BJ, Mattison, DR and Sipes, GI (1990d) The role of epoxidation in 4-vinyl cyclohexene-induce ovarian toxicity. Toxicol Appl Pharmacol 105, 371-381.

Smith, BJ and Sipes IG (1991) Epoxidation of 4-vinylcyclohexene by human hepatic microsomes. Toxicol Appl Pharmacol 109, 367-371.

Smyth HF, Carpenter, CP, Weil, CS *et al*. (1962) Range finding toxicity data: List VI. Am Ind Hyg Assoc J, 23, 95-107.

Smyth HF, Carpenter, CP, Weil, CS *et al*. (1969) Range finding toxicity data: List VII. Am Ind Hyg Assoc J, 30, 470-476.

USEPA (1989) Notice containing the ITC recommendation of 4-VCH to the Priority List and soliciting interested parties for developing a consent order for 4-VCH. 54 FR 51114. December 12, 1989.

Van Duureen, BL, Nelson, N, Orris, L, et. al. (1963) Carcinogenicity of epoxides, lactones and peroxy compounds. Journal of the National Cancer Institute 31, 41-55.

Watabe, T, Hiratsuka, A, Ozawa, N and Isobe, M (1981) A comparative study on the metabolism of d-limonene and 4-vinylcyclohex-1-ene by hepatic microsomes. Xenobiotica 11, 333-344.

Weschler, C.; Hodgson, A.T.; Wooley, J.D. (1992): Indoor Chemistry: Ozone, Volatile Organic Compounds, and Carpets. Environ. Sci. Technol. 26: 2371-2377.

Yalkowsky, S.H. (2003) Aqueous Solubility Data. CRC Press LLC Boca Raton, Forida p. 509

IUCLID

Data Set

Existing Chemical	ID: 100-40-3
CAS No.	100-40-3
EINECS Name	4-vinylcyclohexene
EC No.	202-848-9
Molecular Formula	C8H12
Memo:	4-VCH dataset prepared by Experien Health Sciences Inc.
Printing date:	10-JUL-2006
Revision date:	
Date of last Update:	**10-JUL-2006**
Number of Pages:	**100**
Chapter (profile):	Chapter: 1.7, 1.8.1, 1.10, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6.1, 2.7, 2.8, 3.1.1, 3.1.2, 3.3.1, 3.3.2, 3.5, 3.7, 4.1, 4.2, 4.3, 4.5.1, 4.5.2, 4.6.3, 5.0, 5.1.1, 5.1.2, 5.1.3, 5.2.1, 5.2.2, 5.4, 5.5, 5.6, 5.7, 5.8.1, 5.8.3, 5.10
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.7 Use Pattern

Type: industrial
Category: Chemical industry: used in synthesis

Remark: An intermediate chemical used to produce styrene, flame retardants, fragrances, solvents, polyolefin products, and specialty chemicals such as vinylcyclohexene diepoxide.
31-MAY-2006 (31) (69)

Type: industrial
Category: Chemical industry: used in synthesis

Remark: A precursor in the production of flame retardants and an intermediate in the synthesis of hot melt adhesives and specialty chemicals.
23-MAR-2006 (17)

Type: industrial
Category: Chemical industry: used in synthesis

Remark: An intermediate chemical isolated during the production of Vinylnorbornene to produce ethylidene norborene. The 4-vinylcylcohexene is inadvertently generated and a portion is isolated and converted to 4-vinylcyclohexene monoepoxide or diepoxide, or is incinerated.
23-MAR-2006 (10)

Type: industrial
Category: Chemical industry: used in synthesis

Remark: An intermediate chemical generated during the trimerization of butadiene to cyclododecatriene in the production of dodecanedioic acid. The 4-vinylcylcohexene is a co-product of the process and is either recycled for use as a catalyst solvent, sold, or disposed of by burning in a boiler as fuel.
23-MAR-2006 (10)

Type: industrial
Category: Chemical industry: used in synthesis

Remark: A byproduct generated unintentionally during the production of styrene-butadiene (SB) rubber, SB latex, and polybutadiene rubber products and then subsequently recovered along with styrene for recycling / reuse in the process.
23-MAR-2006 (10)

1.8.1 Occupational Exposure Limit Values

Type of limit: TLV (US)
Limit value: .1 other: ppm

Remark: A3: Confirmed animal carcinogen with unknown relevance to humans.
Excursion Limit Recommendation: Excursions in worker exposure levels may exceed three times the TLV-TWA for no more than a total of 30 min during a work day, and under no circumstances should they exceed five times the TLV-TWA, provided that the TLV-TWA is not exceeded
Reliability: (4) not assignable
Secondary literature.
27-MAR-2006 (2)

Type of limit: other: 8 hr TWA
Limit value: 5 other: ppm

Reliability: (4) not assignable
Secondary literature.
10-JUL-2006 (3)

1.10 Source of Exposure

Source of exposure: Human: exposure by production
Exposure to the: Substance

Remark: 4-Vinylcyclohexene (4-VCH, a dimer of 1,3-butadiene) is present in process streams associated with the refining of crude butadiene for the production of commercial grade 1,3-butadiene. Workers can be exposed to fugitive emissions from process equipment, as well as during line clearing and equipment maintenance and repair activities. The concentration of 4-VCH in the primary process streams has been reported as follows:

- From 10 to 4,600 ppm/w in crude butadiene streams
- 50 to 2,200 ppm/w in refined product streams
- 0.025% to 100% in purge streams.

Purge streams were either incinerated, burned as boiler fuel, or hydrotreated to destroy nearly all the 4-VCH; or they were blended into gasoline or fuel oil.
23-MAR-2006 (9)

Source of exposure: Environment: exposure from processing
Exposure to the: Substance

Remark: In a survey conducted prior to 1989 sponsored by the Effluent Guidelines Division of the U.S. EPA, 4-Vinylcyclohexene was detected at waste water treatment facilities at 2 organics & plastics plants, 6 rubber

processing plants, and 7 publicly owned treatment works at the following concentrations, respectively:

- 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

31-MAY-2006 (62)

Source of exposure: Environment: exposure from production
Exposure to the: Substance

Remark: 4-Vinylcylcohexene (4-VCH) is released into the air as fugitive emissions during the production of 1,3-butadiene and the on-purpose production of 4-VCH, and during downstream processing as a chemical intermediate.

23-MAR-2006 (9)

Source of exposure: Environment: exposure from production
Exposure to the: Substance

Remark: 4-Vinylcylcohexene (4-VCH) is released into plant process sewers and sent to plant waste treatment facilities where it destroyed prior to leaving the site. In 1990, 1 company representing 1 site did report releasing 35 lbs/year after on-site treatment.

23-MAR-2006 (9)

Source of exposure: Human: indirect exposure
Exposure to the: Substance

Remark: 4-Vinylcylcohexene (4-VCH) may be present in styrene / butadiene / acrylonitrile (SBA) copolymers used as a coating for food packaging. The concentration of 4-VCH in the wet latex is capped by the U.S. Food and Drug Administration at 200 ppm. Leaching into food has not been described.

23-MAR-2006 (65)

2.1 Melting Point

Value: = -108.9 degree C

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
Secondary literature (handbook or compilation of data).
27-APR-2006 (35)

2.2 Boiling Point

Value: = 128 degree C

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
Secondary literature (handbook or compilation of data).
27-APR-2006 (35)

2.3 Density

Type: density
Value: = .8299 g/cm^3

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
Secondary literature (handbook or compilation of data).
27-APR-2006 (35)

2.4 Vapour Pressure

Remark: Value = 15.7 mm Hg @ 25 degrees C
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
Secondary literature (handbook or compilation of data).
27-APR-2006 (16)

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: = 3.93

Method: other (measured): no details available
GLP: no data

Remark: The data are cited in the Biodegradation and Bioaccumulation Data of Existing Chemicals based on the CSCL Japan. They have been assigned a reliability rating of 2 because there is insufficient information available on the method and analytical procedures, conducted by the Chemicals Inspection and Testing Institute, Japan.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
Secondary literature (handbook or compilation of data).
27-APR-2006 (43)

2.6.1 Solubility in different media

Solubility in: Water

Remark: Value = 4.622E-04 mol/l @ 25 degrees C.

Value = 5.000E-02 g/l @ 25 degrees C.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
Secondary literature (handbook or compilation of data).
27-APR-2006 (70)

2.7 Flash Point

Value: = 15.9 degree C
Type: open cup

Remark: Original data listed as 289.00 deg Kelvin.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
Secondary literature (handbook or compilation of data).
27-APR-2006 (16)

2.8 Auto Flammability

Value: = 269.9 degree C

Remark: Original data listed as "autoignition temperature" 543.00 deg Kelvin; pressure not specified.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
Secondary literature (handbook or compilation of data).
27-APR-2006 (16)

3.1.1 Photodegradation

Type: air
Light source: Sun light
INDIRECT PHOTOLYSIS
Sensitizer: O3
Conc. of sens.: 700000000000 molecule/cm³
Rate constant: = .000000000000000212 cm³/(molecule * sec)
Degradation: = 50 % after 1.3 hour(s)

Method: other (calculated): AOPWIN version 1.91

Remark: Calculated value using AOPWIN version 1.91, a subroutine of the computer program EPI Suite version 3.12.

Indirect photodegradation, or atmospheric oxidation potential, is based on the structure-activity relationship methods developed by R. Atkinson under the following conditions:

Parameter	Value / Units
Temperature:	25°C
Sensitizer:	ozone
Concentration of Sensitizer:	7.0E11 OH-radicals/cm3 (Atkinson and Carter, 1984)

The half-life of 4-Vinylcyclohexene, based on a 12-hour day, is 0.11 days or 1.3 hours. The half-life is normalized to a 12-hour day because atmospheric oxidation reactions only take place in the presence of sunlight.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.
10-JUL-2006 (5) (21) (39)

Type: air
Light source: Sun light
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1500000 molecule/cm³
Rate constant: = .00000000008934 cm³/(molecule * sec)
Degradation: = 50 % after 1.4 hour(s)

Method: other (calculated): AOPWIN version 1.91

Remark: Calculated value using AOPWIN version 1.91, a subroutine of the computer program EPI Suite version 3.12.

Indirect photodegradation, or atmospheric oxidation potential, is based on the structure-activity relationship methods developed by R. Atkinson under the following conditions:

Parameter	Value / Units

Temperature: 25°C
Sensitizer: OH- radical
Concentration of Sensitizer: 1.5E6 OH- radicals/cm3
(Leifer, 1993; Mount and Eisele, 1992)

The half-life of 4-Vinylcyclohexene, based on a 12-hour day, is 0.12 days or 1.4 hours. The half-life is normalized to a 12-hour day because atmospheric oxidation reactions only take place in the presence of sunlight.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.

10-JUL-2006 (4) (21) (34) (44)

3.1.2 Stability in Water

Type: abiotic

Method: other (calculated): calculated using HYDROWIN version 1.67

Remark: Calculated values using HYDROWIN version 1.67, a subroutine of the computer program EPI Suite version 3.12.

Result: Due to a lack of hydrolysable functional groups, 4-VCH would not be expected to hydrolyze appreciably in an aqueous environment. The hydrolysis half-life is estimated to be greater than a year.

The structure of 4-vinylcyclohexene is that of an alicyclic hydrocarbon, a class of molecule not considered to be water reactive at environmental pH values. HYDROWIN could not calculate a hydrolysis rate for 4-Vinylcyclohexene, an expected result.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.

10-JUL-2006 (21) (41)

3.3.1 Transport between Environmental Compartments

Type: fugacity model level I
Media: other: air - soil - sediment - water
Method: other: LEVEL I version 3.00, a Fugacity-based model

Remark: Physicochemical data used in the calculation:

Parameter	Value / Units
Molecular Weight	108.18 g/moleTemperature 25°CLog Kow 3.93Water
Solubility	50 mg/l
Vapor Pressure	2102 Pa
Melting Point	-108.9 degrees C

The program models environmental partitioning of a release of 100000 kg of 4-VCH under instaneous equilibrium conditions using the Mackay Level I Fugacity model. Sediment is considered to be part of the water column.

Additional partitioning was calculated:
Sediment: 0.0181%

Result: Air: 99.1% (Fugacity Model Level I)
Water: 0.108% (Fugacity Model Level I)
Soil: 0.814% (Fugacity Model Level I)
Biota: 4.59E-05% (Fish - Fugacity Model Level I)

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Reliability: (2) valid with restrictions
This robust summary has a reliability rating of 2 because the distribution data are modeled.

10-JUL-2006 (8)

Type: fugacity model level III
Media: other: air - soil - sediment - water
Method: other: calculated using LEV3EPI

Remark: Physicochemical data used in the calculation:

Parameter	Value / Units
Molecular Weight	108.18 g/moleTemperature 25°CLog Kow 3.93Water
Solubility	50 mg/l
Vapor Pressure	15.77 mm Hg
Soil Koc	3.49e+03 (calculated by model)

The program models environmental partitioning under steady-state conditions using the Mackay Level III Fugacity model. The standard emission rates to air, water and soil are: 1000 kg/hr to air, 1000kg/hr to water and 1000 kg/hr soil. Sediment is considered to be part of the water column.

Result: Air: 0.52% (Fugacity Model Level III)
Water: 35.0% (Fugacity Model Level III)
Soil: 60.6% (Fugacity Model Level III)

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.
10-JUL-2006 (21)

3.3.2 Distribution

Media: other: wastewater - surface water
Method: other (calculation): STPWIN

Remark: Percent removal in a wastewater treatment facility STPWIN is a subroutine of the computer program EPI Suite version 3.12.

The predicted removal in a wastewater treatment facility having a primary, aeration and settling tank is 95%.

Physicochemical data used in the calculation:

Parameter	Value / Units
Molecular Weight	108.18 g/moleWater
Solubility	50 mg/l
Vapor Pressure	15.77 mm Hg
Henry's Law Constant	0.0448 atm-m3/mole
Octanol-water partition coefficient	1.83
Air-water partition coeffficient (Kow)	8511 (calculated by program)
Log Kow	3.93
Biomass to water parttion coefficient	1703 (calculated by program)
Temperature	25°C

The program models environmental partitioning under instaneous steady-state conditions using the Toronto Model developed by McKay and colleagues as described by Clark et. al.

The primary mode of removal was aeration off gas (78%) followed by partitioning to sludge (15%). Biodegradation accounted for 0.1% of total removal.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.
10-JUL-2006 (12) (21)

Media: water - air
Method: other (measurement): HENRYWIN version 3.10

Remark: Calculation of Henry's Law constant using HENRYWIN version 3.10 a subroutine of the computer program EPI Suite version 3.12.
Result: Will volatilize from water.

Based on a water solubility of 50 mg/L and a vapor pressure of 15.7 mm Hg (at 25 degree C), a Henry's law constant of 0.155 atm-m3/mol or 1.57E+04 Pa-m3/mole is estimated.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The data are based on measured water solubility and measured vapour pressure data using an accepted calculation method.
10-JUL-2006 (21) (36) (37)

Media: water - air
Method: other (calculation): HENRYWIN version 3.10

Result: Will volatilize from water.

0.044 atm-m3/mole at 25°C or 4.54E+03 Pa-m3/mole at 25°C.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (4) not assignable
The data was part of a compilation of values for various compounds derived by USEPA OPPT from various publications and articles.
10-JUL-2006 (21)

Media: water - air
Method: other (calculation): Volatilization from Water sub-routine

Remark: calculated by Volatilization from Water a subroutine of the computer program EPI Suite version 3.12.
Result: 3.1-hours from river
4.1 days from lake

The volatilization half-life of 4-vinylcyclohexene from a model river (water depth of 1 meter, current velocity of 1 m/sec, and wind velocity of 3 m/sec) and model lake (water depth of 1 meter, current of 0.05 m/sec, and wind velocity of 0.5 m/sec) was estimated.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.
10-JUL-2006 (21)

Media: water - soil
Method: other (calculation): PCKOCWIN version 1.66

Remark: Calculated value using PCKOCWIN version 1.66 a subroutine of the computer program EPI Suite version 3.12.
Result: Moderate adsorption to soil predicted.

Koc (estimated) = 518

Method based on the Sabljic molecular connectivity method with correction factors added to PCKOCWIN version 1.66 a subroutine of the computer program EPI Suite version 3.12. Log Koc was calculated using SMILES notation.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.
10-JUL-2006 (21) (38) (54) (55)

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge
Concentration: 30 mg/l related to DOC (Dissolved Organic Carbon)
100 mg/l related to Test substance
Contact time: 28 day(s)
Degradation: = 0 % after 28 day(s)
Result: other: not readily biodegradable
Kinetic: 28 day(s) = 0 %
Control Subst.: Aniline
Kinetic: 7 day(s) > 40 %
14 day(s) > 60 %

Method: OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (1) valid without restriction
Report not available for review and there is only limited information available on test parameters. Guideline study, results cited in recognised data compendium.
10-JUL-2006 (11)

Type: aerobic
Result: other: not readily biodegradable

Method: other: calculated using BIOWIN version 4.02

Remark: Calculation of biodegradation and the timeframe for Primary and Ultimate biodegradation using BIOWIN version 4.02, a subroutine of the computer program EPI Suite version 3.12 as described by Howard, et. al. in 1994.

BIOWIN contains six models (linear regression (BIOWIN 1), non-linear regression (BIOWIN 2), ultimate degradation to CO2 and H2O (BIOWIN 3), primary degradation (BIOWIN 4), linear regression estimate of the probability of passing the OECD 301C / MITI-1 ready biodegradation test (BIOWIN 5) and non-linear regression estimate of the probability of passing the OECD 301C / MITI-1 ready biodegradation test (BIOWIN 6).

BIOWIN 1 - "Biodegrades Fast"
BIOWIN 2 - "Biodegrades Fast"
BIOWIN 3 - "Weeks"
BIOWIN 4 - "Days-Weeks"
BIOWIN 5 - "Does not biodegrade fast"
BIOWIN 6 - "Biodegrades fast"

According to the USEPA, BIOWIN 6 is better predictor of whether a chemical will pass or fail the OECD 301C / MITI-1 ready biodegradation test than BIOWIN 5.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.
10-JUL-2006 (21) (28)

3.7 Bioaccumulation

Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 56 day(s) at 25 degree C
Concentration: 100 mg/l
BCF: = 83 - 211

Method: OECD Guide-line 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
GLP: yes

Remark: Low bioconcentration.

Lipid content of test fish ranged from 2 - 6% with a mean of 4.1%. An improved apparatus for volatile substances was used.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (1) valid without restriction
Report not available for review and there is only limited

information available on test parameters. Guideline study, results cited in recognised data compendium.

10-JUL-2006 (11)

Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 56 day(s) at 25 degree C
Concentration: 10 mg/l
BCF: = 110 - 208

Method: OECD Guide-line 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
GLP: yes

Remark: Low bioconcentration.

Lipid content of test fish ranged from 2 - 6% with a mean of 4.1%. An improved apparatus for volatile substances was used.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (1) valid without restriction
Report not available for review and there is only limited information available on test parameters. Guideline study, results cited in recognised data compendium.

10-JUL-2006 (11)

Method: other: calculated using BCFWIN version 2.15

Remark: The potential for bioaccumulation of 4-Vinylcyclohexene in the aquatic environment is expected to be low.

The bioconcentration factor (BCF) is calculated from the octanol-water partition coefficient (Log Kow) using an atom/fragment contribution method similar to that described for KOWWIN as documented in a publication for the USEPA by Meylan, et. al. in 1997.

A log BCF of 2.33 (BCF = 211.9) was calculated.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.

10-JUL-2006 (21) (40)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: other: model
Species: other: freshwater fish
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50: = 1.23 - calculated

Method: other: calculated using ECOSAR version 0.99h

Remark: Calculated value using ECOSAR version 0.99h, a subroutine of the computer program EPI Suite version 3.12.

Physicochemical data used in the calculation:

Parameter	Value / Units
Molecular Weight	108.18 g/mole
Log Kow	3.93
Water Solubility	50 mg/l
Melting Point	-108.90 deg C
SMILES Notation	C(=CCCC1C=C)C1

ECOSAR utilized the SMILES notation to select the appropriate compound class. The "Neutral Organics" structure-activity relationship class was selected. Mortality data to fathead minnows measured by Veith, et. al. (1983) for industrial chemicals having narcotic effects was utilized by ECOSAR to determine the freswater fish 96-hr LC50 for 4-Vinylcyclohexene.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.

31-MAY-2006 (21) (67)

Type: semistatic
Species: Oryzias latipes (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50: = 17 - measured/nominal

Method: other: Japanese Industrial Standard *JIS K 0102-1986-71)
GLP: yes

Test condition: 25 deg C, 48-hr exposure of 10 fish under static to semi-static conditions at each concentration level.

Fish were disinfected and acclimatized according to established protocol and analyzed for mercury content.

The measured 48-hr LC50 value was estimated by the Doudoroff method or the Probit method.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
Report not available for review and there is only limited information available on test parameters. Guideline study, results cited in recognised data compendium.
10-JUL-2006 (11)

Species: Oryzias latipes (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50: = 4.6 - measured/nominal

Test condition: These data are based on emasured values.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The data are cited in the Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
10-JUL-2006 (42)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: other: model
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50: = 1.51 - calculated

Method: other: calcualted using ECOSAR version 0.99h

Remark: Calculated value using ECOSAR version 0.99h, a subroutine of the computer program EPI Suite version 3.12.

Physicochemical data used in the calculation:

Parameter	Value / Units
Molecular Weight	108.18 g/mole
Log Kow	3.93
Water Solubility	50 mg/l
Melting Point	-108.90 deg C
SMILES Notation	C(=CCCC1C=C)C1

ECOSAR utilized the SMILES notation to select the appropriate compound class. The "Neutral Organics" structure-activity relationship class was selected by the program. Mortality data to Daphnia magna measured by Hermans, et. al. (1984) for chemical mixtures having anesthetic effects was utilized by ECOSAR to determine the freswater Daphnia 48-hr LC50 for 4-Vinylcyclohexene.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.
10-JUL-2006 (21) (25)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50: = 1.9 - calculated

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The data are cited in the Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
10-JUL-2006 (42)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Pseudokirchneriella subcapitata (formely known as Selenastrum capricornutum)
Endpoint: other: area under growth curve
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50: > 14 - measured/nominal

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The data are cited in the Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
10-JUL-2006 (42)

Species: other algae: Pseudokirchneriella subcapitata (formely known as Selenastrum capricornutum)
Endpoint: other: area under growth curve
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:**
NOEC: = 7.7 - measured/nominal

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The data are cited in the Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
10-JUL-2006 (42)

Species: other algae: Pseudokirchneriella subcapitata (formely known as Selenastrum capricornutum)
Endpoint: other: growth
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50: > 14 - measured/nominal

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The data are cited in the Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
10-JUL-2006 (42)

Species: other algae: model, freshwater green algae
Endpoint: other: growth
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50: = 1.05 - calculated

Method: other: calculated using ECOSAR version 0.99h

Remark: Calculated value using ECOSAR version 0.99h, a subroutine of the computer program EPI Suite version 3.12.

Physicochemical data used in the calculation:

Parameter	Value / Units
Molecular Weight	108.18 g/mole
Log Kow	3.93
Water Solubility	50 mg/l
Melting Point	-108.90 deg C
SMILES Notation	C(=CCCC1C=C)C1

ECOSAR utilized the SMILES notation to select the appropriate compound class. The "Neutral Organics" structure-activity relationship class was selected by the program. Growth data for green algae measured by Calamari, et. al. (1983) for selected chlorobenzenes, Galassi and Vighi (1981) for volatile substances and USEPA (1991) data from PMN submissions were utilized by ECOSAR to determine the freswater green algae 96-hr EC50 for 4-Vinylcyclohexene.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.
10-JUL-2006 (7) (21) (22)

Species: other algae: model, freshwater green algae
Endpoint: other: growth (chronic value, ChV)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50: = .32 - calculated

Method: other: calculated using ECOSAR version 0.99h

Remark: Calculated value using ECOSAR version 0.99h, a subroutine of the computer program EPI Suite version 3.12.

Physicochemical data used in the calculation:

Parameter	Value / Units
Molecular Weight	108.18 g/mole
Log Kow	3.93
Water Solubility	50 mg/l
Melting Point	-108.90 deg
CSMILES Notation	C(=CCCC1C=C)C1

ECOSAR utilized the SMILES notation to select the appropriate compound class. The "Neutral Organics" structure-activity relationship class was selected. Growth data for green algae measured by Calamari, et. al. (1983) and USEPA (1991) data from PMN submissions were utilized by ECOSAR to determine the freshwater green algae 96-hr chronic value (ChV) for 4-Vinylcyclo-hexene.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.

31-MAY-2006 (7) (21) (22) (64)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

Species: other: model, freshwater fish
Endpoint: other: survival / growth
Exposure period: 30 day(s)
Unit: mg/l **Analytical monitoring:**
Chronic Value (ChV) :
= .22 - calculated

Method: other: calculated using ECOSAR version 0.99h

Remark: Calculated values using ECOSAR version 0.99h, a subroutine of the computer program EPI Suite version 3.12.

Physicochemical data used in the calculation:

Parameter	Value / Units
Molecular Weight	108.18 g/mole
Log Kow	3.93
Water Solubility	50 mg/l
Melting Point	-108.90 deg
CSMILES Notation	C(=CCCC1C=C)C1

ECOSAR utilized the SMILES notation to select the appropriate compound class. The "Neutral Organics" structure-activity relationship class was selected. USEPA (1991) survival / growth data from PMN submissions for freshwater fish were utilized by ECOSAR to determine the freswater fish 30-day chronic value (ChV) for 4-Vinylcyclo-hexene.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.
31-MAY-2006 (21) (63)

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species: other: model, Daphnia magna
Endpoint: other: reproduction
Exposure period: 16 day(s)
Unit: mg/l **Analytical monitoring:**
EC50: = .18 - calculated

Method: other: calculated using ECOSAR version 0.99h

Remark: Calculated values using ECOSAR version 0.99h, a subroutine of the computer program EPI Suite version 3.12.

Physicochemical data used in the calculation:

Parameter	Value / Units
Molecular Weight	108.18 g/mole
Log Kow	3.93
Water Solubility	50 mg/l
Melting Point	-108.90 deg
CSMILES Notation	C(=CCCC1C=C)C1

ECOSAR utilized the SMILES notation to select the appropriate compound class. The "Neutral Organics" structure-activity relationship class wasselected. Reproductive data to Daphnia magna measured by Hermans, et. al. (1984) for chemical mixtures having anesthetic effects was utilized by ECOSAR to determine the freswater Daphnia 15-day EC50 for 4-Vinylcyclohexene.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.
31-MAY-2006 (21) (25)

Species: Daphnia magna (Crustacea)
Endpoint: other: reproduction
Exposure period: 21 day(s)
Unit: mg/l **Analytical monitoring:**
EC50: = .92 - measured/nominal

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The data are cited in the Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
10-JUL-2006 (42)

Species: Daphnia magna (Crustacea)
Endpoint: other: reproduction
Exposure period: 21 day(s)
Unit: mg/l **Analytical monitoring:**
EC50: = .23 - measured/nominal

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The data are cited in the Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. This robust summary has a reliability rating of 2 because there is insufficient information available on the method and analytical procedure.
10-JUL-2006 (42)

TERRESTRIAL ORGANISMS

4.6.3 Toxicity to Soil Dwelling Organisms

Species: other: earthworm
Endpoint: other: mortality
Exposure period: 14 day(s)
Unit: other: ppm (dry soil wt)
LC50: = 169.3 - calculated

Method: other: calculated using ECOSAR version 0.99h

Remark: Calculated values using ECOSAR version 0.99h, a subroutine of the computer program EPI Suite version 3.12.

Physicochemical data used in the calculation:

Parameter	Value / Units
Molecular Weight	108.18 g/mole
Log Kow	3.93
Water Solubility	50 mg/l
Melting Point	-108.90 deg
CSMILES Notation	C(=CCCC1C=C)C1

ECOSAR utilized the SMILES notation to select the appropriate compound class. The "Neutral Organics" structure-activity relationship class was selected. Mortality data to Eisenia fetida and other earthworm species was measured by Neuhauser, et. al. (1985, 1986) for selected organic chemicals was utilized by ECOSAR to determine the earthworm 14-day LC50 for 4-Vinylcyclohexene.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
The value was calculated based on chemical structure as modeled by EPI Suite. This robust summary has a reliability rating of 2 because the data are modeled.
10-JUL-2006 (21) (45) (46)

5.0 Toxicokinetics, Metabolism and Distribution

Type: Toxicokinetics
Species: other: comparative distribution and metabolism studies in rats and mice

Method: Female B6C3F1 mice (17-23 g) and Fischer 344 rats (175-250 g) were fasted overnight, administered 4-VCH (containing 14C-labelled material) by gavage at 400 mg/kg body weight and subsequently sacrificed at selected time-points (1-48 hr post-dose). The amount of radioactivity given (4-45 uCi/mouse; 4-80 uCi/rat) was varied to maximise detection of 14C-4-VCH in the ovary at the later time-points used.

Excreta (urine, feces, exhaled air) were collected with the animals housed in glass metabolism cages, with subgroups of animals (n = 3-4) sacrificed (carbon dioxide) at pre-selected intervals (hourly or 4 hourly, up to 48 hr) for necropsy. Major organs were weighed, sampled and stored at -20 degrees C prior to sample oxidation in duplicate and quantitation of total 14C-carbon dioxide by liquid scintillation counting.

Radioactivity present in exhaled air (volatile fraction trapped using 2-methoxyethyl ether, exhaled carbon dioxide using Carbosorb/ethylene glycol; arranged in series) or urine was subject to direct liquid scintillation counting, while 14C in feces was digested with potassium hydroxide prior to sample oxidation.

In other studies blood, muscle, skin, adipose tissue and ovary (selected on the basis of results for disposition studies, described above) from female rats and mice given 4-VCH (400 mg/kg body weight, i.p.) were sampled (1-8 hr post-treatment), snap frozen (liquid nitrogen) and stored on dry ice prior to processing (homogenisation/hexane extraction; decane internal standard) and analysis for 4-vinylcyclohexene by GC-FID. Tissue recovery studies demonstrated an extraction and recovery efficiency of 80-89%, with a detection limit of at least 0.05 ug 4-VCH/g tissue for ovary.

The time-course for appearance of 4-vinylcyclohexene-1,2-epoxide (4-VCH 1,2-EP) and 4-vinylcyclohexene-7,8-epoxide (4-VCH 7,8-EP) in blood was investigated in female rats and mice given 4-VCH at 800 mg/kg body weight by i.p. injection. Animals were sacrificed 0.5, 1, 2, 4 or 6 hr post-dose, blood (cardiac puncture) collected into heparinsed tubes and the epoxides quantified by capillary GC after hexane extraction (cis-cyclodecane internal standard; detectable at 1.25 nmol/sample and above).

NADPH-dependent metabolism of 4-VCH to 4-VCH 1,2-EP by hepatic

microsomal fractions from rat and mouse was investigated in vitro (pH 7.5) in screw capped vials in the presence of 3,3,3-trichloropropene oxide (inhibitor of microsomal epoxide hydrolase). Samples were analyzed using capillary GC (as for blood samples, above).

Statistically significant differences between means were investigated using Student's t-test.

Result: Elimination of radioactivity associated with oral administration of a single oral dose of 4-VCH (400 mg/kg bwt) was virtually complete in the mouse within 24 hr, whereas rats required 48 hr. The main routes of excretion of 4-VCH-derived radioactivity were urine and expired air, with small amounts in feces and the tissues:

	Percent total dose	
Parameter	Rat	Mouse
Time (hr)	48	24
Urine	52.1	57.7
Expired organics*	36.0	31.4
Feces	9.6	3.1
Tissues	2.4	1.8
Cage wash	0.6	2.9
Recovery	100.7	96.9

* negligible amounts of 14C-carbon dioxide excreted.

Tissue distribution studies generally demonstrated retention of only trace amounts (up to 1%) of 4-VCH derived radioactivity in skin, muscle, liver and blood from both species 24 hr post-dose, with approx. 3% rat adipose tissue (trace amounts in mouse). Levels in rat and mouse ovary were minimal (<0.02% of dose in rat, 0.03% or less in mouse). Comment: although percent retention of total administered dose in ovary was low, the peak concentration of [14C]-derived 4-VCH equivalents (0.7-1.3 nmol/mg tissue, both species) was comparable to that found in liver (1.1-1.6 nmol/mg).

Investigations into tissue distribution of the parent substance (analysed as 4-VCH) showed that levels were greatest in adipose tissue, which peaked in the mouse 1-2 hr post-dose (approx. 4 nmol 4-VCH/mg tissue; negligible by 6 hr) but continued to accumulate in the rat until at least 6 hr after oral administration (approx. 6 nmol 4-VCH/mg tissue). The concentration 4-VCH in other tissues was one tenth or less than that of adipose tissue with negligible amounts remaining 6-8 hr post-dose, with slightly higher values obtained for rats compared to mice.

Blood analyses for 4-VCH 1,2-EP indicated a peak in mice of 41 nmol/ml (2 hr post-dose) but 4-VCH 7,8-EP was absent (limit of detection 2.5 nmol/ml). Neither metabolite was detectable in blood from rats given 800 mg/kg 4-VCH by gavage.

Conversion of 4-VCH to epoxide metabolites by hepatic

microsomal fractions in vitro revealed that formation of the 1,2-epoxide was approx. 6.5-fold greater for mouse than for rat when expressed on the basis of mg microsomal protein, or 4-fold greater when expressed as specific activity (per nmol cytochrome P450):

	-------- 4-VCH 1,2-EP --------	
Species	nmol/min/mg	nmol/min/nmolP450
Rat	1.4	1.6
Mouse	9.1**	6.6**

** p<0.05

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: These studies demonstrate that urine and exhaled air are the main routes of excretion of 4-VCH following oral (gavage) administration to female rats and mice. Tissue distribution studies indicated generally low levels of retention of 4-VCH derived material in both species, with slight preferential partitioning in adipose tissue but not ovary. Mice more rapidly metabolize 4-VCH to the 1,2-epoxide than the rat.

Reliability: (2) valid with restrictions
Study available for review. Non-guideline experimental study. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (57)

Type: Toxicokinetics
Species: other: comparative distribution and metabolism studies in rats and mice

Method: Appearance of 4-VCH 1,2 epoxide (4-VCH 1,2-EP) in blood after treatment with 4-VCH was investigated in groups of female B6C3F1 mice (Harlan Spargue-Dawley, Indianapolis, IN; age 28 d; n = 3-4 per treatment) administered 0, 100, 400 or 800 mg 4-VCH/kg body weight by single i.p. injection in corn oil (2.5 ml/kg body weight). Animals were sacrificed 2 hr post-dose (carbon dioxide), blood collected by cardiac puncture and analyzed for 4-VCH 1,2-EP by capillary GC after hexane extraction (cis-cyclodecane internal standard).

In other studies the time course for removal of 4-VCH (2.7 mmol/kg) or 4-VCH 1,2-EP (0.49 mmol/kg) from blood was investigated in female mice after i.p. administration. Groups of animals (n = 3-4 per time point) were sacrificed 0, 15, 30, 60 120, 180 and 240 minutes post-dose, blood collected (cardiac puncture) and analysed for 4-VCH 1,2-EP (as above) and 4-VCH (GC-FID after hexane extraction with decane internal standard). The AUC was estimated graphically.
Comment: dose selection was based on ovarian toxicity studies performed by these authors and reported in section 5.8.3 of these Robust Summaries.

The impact of chloramphenicol (an inhibitor of cytochrome

P-450 mediated epoxidation; 0, 50, 100, 200 or 300 mg/kg body weight in saline) administered by i.p. injection 1 hr prior to 4-VCH treatment (800 mg/kg body weight, i.p. in corn oil) on the appearance of 4-VCH 1,2-EP in blood was also investigated in female mice (n = 4 per group). Hepatic microsomal fractions were also prepared from control (saline, i.p.) or chloramphenicol (200 mg/kg, i.p.) treated female mice 1 hr post-treatment, and NADPH-dependent conversion of 4-VCH (1 mM) to 4-VCH 1,2-EP followed in vitro (pH 7.5) in screw capped vials in the presence of 3,3,3-trichloropropene oxide (inhibitor of microsomal epoxide hydrolase). Samples were analysed using capillary GC (as for blood samples, above).

Dose-response curves were obtained by non-linear regression, and significant differences between curves analyzed using the sum of squares of the two data sets under comparison and as a single pool to calculate an F value. Student's t-test was used to determine the significance of differences between group means while multiple comparisons used one-way ANOVA and the Newman-Kuels range test.

Result: The concentration of 4-VCH 1,2-EP in blood increased in a dose-related manner 2 hr after i.p. administration of 4-VCH to female mice:

Dose 4-VCH (mg/kg bw)	4-VCH 1,2-EP (nmol/ml blood)
0	0.0
100	3.5
400	27
800	42

Graphical results showed that i.p. administration of overtly ovotoxic doses of 4-VCH (2.7 mmol/kg bw) or 4-VCH 1,2-EP (0.49 mmol/kg bw) resulted in clear differences in blood concentration/time profiles in female mice i.e.

- from 5-15 min post-treatment, the blood concentration of 4-VCH 1,2-EP (approx. 100 nmol/ml blood) was much greater than that of 4-VCH (approx. 10 nmol/ml blood);
- from 30-120 min post-treatment, the concentration of 4-VCH (max. approx. 25 nmol/ml blood and declining but detectable thereafter) was much greater than that of 4-VCH 1,2-EP (<10 nmol/ml blood at 30 min, undetectable from 60 min);

However the AUCs for blood concentration were comparable (50 and 26 nmol/ml*hr for 4-VCH or 4-VCH 1,2-EP treated mice, respectively).

Administration of a single dose of chloramphenicol 1 hr prior to treatment with 4-VCH inhibited formation of 4-VCH 1,2-EP and its appearance in blood in a dose-dependent manner:

Chloramphenicol (mg/kg bw)	4-VCH 1,2-EP (nmol/ml blood)

0	100%
50	73% *
100	50% *
200	40% *
300	45% *

* P<0.05
(Values obtained by interpolation from graphical data.)

Conversion of 4-VCH to 4-VCH 1,2-EP by hepatic microsomal fractions from female mice in vitro was also decreased after pre-treatment with chloramphenicol (200 mg/kg bw):

	4-VCH 1,2-EP (nmol/min/mg microsomal protein)	Cytochrome P-450 (nmol/mg protein)
Control (saline)	8.8	0.88
Chloramphenicol	2.7 **	0.75 *

* P<0.05
** P<0.01
Comment: part of this decrease may reflect a small but significant reduction in microsomal P-450 content.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Results from these investigations demonstrate differences in the toxicokinetics of 4-VCH and 4-VCH 1,2-EP in mouse, with the latter exhibiting more rapid uptake and clearance from blood after i.p. administration than the parent substance. Metabolism of 4-VCH to 4-VCH 1,2-EP in vivo and by hepatic microsomal fractions in vitro was inhibited by pre-treatment of mice with chloramphenicol (an epoxide hydrolase inhibitor).

Reliability: (2) valid with restrictions
Study available for review. Non-guideline experimental study. Well reported methods and results, acceptable for evaluation.
10-JUL-2006 (58)

In Vitro/in vivo: In vitro
Type: Distribution
Species: other: rats and mice

GLP: yes

Method: Air:tissue partition coefficients for 4-VCH (99% pure) and its 1,2 epoxide (>95% pure) and 7,8 epoxide (>97% pure) metabolites were determined using a vial equilibration technique (Gargas et al (1989) Toxicol. Appl. Pharmacol. 98, 87-99) and blood, liver, lung ovary, fat and muscle preparations obtained from untreated female Crl:CD rats (body weight = 200-300 g) and untreated female B6C3F1 mice (body weight = 26-37 g).

Incubations (2-4 replicates per tissue, dependent on amount of sample available) were conducted in vials pre-treated with silicone-based glass deactivator (to minimize adsorption of

test substance) containing enzyme deactivated tissue homogenate, pre-equilibrated to 37 degrees C. Experiments (37 degrees with mixing, duration 20-180 min) were initiated by removal of 0.5-1.0 ml of headspace air and its replacement with an equivalent of vaporized test substance (750-2000 ppm). 1,1,1-Trichloropropene oxide was added to vials containing epoxide substrates to prevent expression of any residual epoxide hydrolase activity.

Headspace samples were taken at regular intervals and analyzed by GC-FID. Partition coefficients were calculated using Microsoft Excel.

Comment: no equilibrium was reached in the test systems (presumed due to adsorption of test substances to vial wall) with the derived partition coefficients changing with time (as the concentration in the headspace altered). This was corrected by plotting the apparent partition coefficients against time, and back-extrapolating to time zero using linear regression.

Comment: partition coefficients for 4-VCH diepoxide could not be measured since it was insufficiently volatile for the methods used in this study.

Result: 4-VCH

The solubility of 4-VCH in mouse tissues and blood was generally slightly higher than the corresponding rat tissue, with the exception of ovary. It was very soluble in fat relative to air.

	Rat	Mouse
Blood:air	17	20
Liver:air	63	88
Lung:air	26	52
Ovary:air	81	41
Fat:air	332	899
Muscle:air	20	47

4-VCH 1,2 epoxide

The solubility of 4-VCH 1,2 epoxide in mouse tissues and blood was generally higher than the corresponding rat tissue, with the exception of ovary. It was very soluble in fat, rat ovary and mouse liver:

	Rat	Mouse
Blood:air	171	291
Liver:air	302	913
Lung:air	149	394
Ovary:air	695	353
Fat:air	2152	6346
Muscle:air	109	302

4-VCH 7,8 epoxide

The solubility of 4-VCH 7,8 epoxide in mouse tissues and blood was generally higher than the corresponding rat tissue, with the exception of liver (greater for rat) and ovary (comparable

for rats and mice). It was very soluble in fat.

	Rat	Mouse
Blood:air	230	113
Liver:air	365	275
Lung:air	244	522
Ovary:air	497	435
Fat:air	1072	4727
Muscle:air	138	208

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Air:tissue partition coefficients for 4-VCH and its 1,2 and 7,8 epoxides in mouse tissues and blood were generally higher than that of the corresponding rat tissue, with the exception of ovary (where the air:tissue partition coefficient was generally higher for the rat). Air:fat partition coefficients were greater than those for other tissues and blood in both species. Tissue/blood solubility of the epoxide metabolites was consistently higher than that of the parent substance in both rats and mice.

Reliability: (2) valid with restrictions
Study available for review. Non-guideline GLP-compliant experimental study. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (32)

In Vitro/in vivo: In vitro
Type: Metabolism
Species: other: rats and mice

Method: Adult female mice (B6C3F1 strain, Harlan Sprague Dawley, Indianapolis, IN; 129/J strain, Jackson Laboratories, Bar Harbor, ME) and F344 rats (Harlan Sprague Dawley) were given 0.1% phenobarbital in drinking water for 6 days or dexamethasone (100 mg/kg body weight, in corn oil) by i.p. injection for 4 days (B6C3F1 mice only). Animals were then sacrificed (cervical dislocation) and the hepatic microsomal fraction isolated from individual rat livers, or from two pooled 2 mouse livers.

In some studies B6C3F1 mice were pre-treated with chloramphenicol sodium succinate (as chloramphenicol base, 200 mg/kg in saline) by i.p. injection (2.5 ml/kg body weight) one hour prior to sacrifice and preparation of the microsomal fraction.

Androsterone hydroxylase and testosterone hydroxylase activities present in hepatic microsomal fractions were quantified using published methods. Hepatic microsomal 4-VCH epoxidase activity (1 mM 4-VCH, 0.1-0.5 mg/ml microsomal protein, NADPH generating system) was determined using capillary GC; under these conditions, no inhibitor of epoxide hydrolase was required. Microsomal fractions from control or

pre-treated rats and mice were used in these studies, with some incubations performed in the presence of antibodies specific to rat- or mouse cytochrome P450 isozymes (anti-rat P450 PB-B, reactive to P450IIB; anti-rat P450PCNb, reactive to P450IIIA; anti-mouse P45015a, reactive to P450IIA).

Microsomal proteins were separated using standard Western blot methods, visualized using horseradish peroxidase (Immuno-Blot assay kit) and immunoreactive material quantified using a Joyce-Lobel laser densitometer.

Student's t-test was used to compare means.

Result: Chloramphenicol pre-treatment of female B6C3F1 mice resulted in a statistically significant loss of testosterone hydroxylation at the 15a (decreased 46%) and 6B positions (62%), consistent with it inhibiting cytochrome P450IIA- and cytochrome P450IIIA-dependent isozymes.

Pre-treatment of B6C3F1 mice with phenobarbital (inducer of P450IIB) increased metabolism of 4-VCH to 4-VCH 1,2-EP approx. 5-fold and hydroxylation of testosterone by approx. 3-5 fold. Pre-treatment with dexamethasone (inducer of P450IIIA) increased 4-VCH epoxidation approx. 3-fold, and testosterone hydroxylation in the 16a and 6B positions by around 2 and 4-fold, respectively.
These findings suggest involvement of cytochrome P450IIB and P450IIIA in metabolism of 4-VCH by female mice.

Pre-treatment of female F344 rats with phenobarbital increased hepatic microsomal 4-VCH epoxidase activity by around 9-fold and androsterone 16B hydroxylation by approx. 47-fold, and support a role for cytochrome P450IIB in the metabolism of 4-VCH.

Pre-incubation of hepatic microsomes from untreated female B6C3F1 mice with anti-rat P450PB-B immunoglobulin G resulted in a 35% decrease in 4-VCH epoxidase activity and a 48% decrease in testosterone-16a-hydroxylase activity (negligible effect on testosterone hydroxylation in other positions). Pre-incubation with anti-rat P450PCNb immunoglobulin G was without effect on epoxidation of 4-VCH while testosterone-6B-hydroxylation was inhibited by 68%. Incubation of control mouse microsomal fractions with anti-rat P45015a immunoglobulin G decreased 4-VCH epoxidase activity by 47%, and testosterone-15a-hydroxylase activity by 86%. These results indicate that cytochrome P450IIA and IIB (but not IIIA) are responsible for 4-VCH epoxidase activity in untreated female mice.

In studies using microsomal fractions from female F344 rats, anti-rat P450PB-B immunoglobulin G decreased hepatic microsomal epoxidation of 4-VCH and androsterone-16B-hydroxylase activity by 33% and 38%, respectively, in control preparations and by 89% and 93% in

phenobarbital-induced fractions, respectively. These findings indicate that cytochrome P450IIB isozymes play a relatively minor role in the metabolism of 4-VCH in untreated female rats, but are induced and responsible for increased metabolism of 4-VCH after phenobarbital treatment.

A role for cytochrome P450IIB in the metabolism of 4-VCH was also demonstrated in studies using strain 129/J mice, which possess low constitutive levels of this isozyme in the liver. In these experiments, expression 4-VCH epoxidase- and testosterone-16a-hydroxylase activities in control female 129/J mice were both around one third lower than those of control female B6C3F1 mice, but both were increased 8 to 9-fold after phenobarbital pre-treatment.

Western blot analysis confirmed that constitutive levels of hepatic cytochrome P450IIB were around 4-fold lower in female 129/J mice relative to female B6C3F1 mice but is inducible in both strains after phenobarbital treatment. Cytochrome P450IIB was undetectable in untreated female F344 rats, but increased after treatment with phenobarbital. Immunoblots obtained using anti-mouse P45015a immunoglobulin showed the presence of a single band (cytochrome P450IIA) in hepatic microsomes from female B6C3F1 mice which was induced following treatment with phenobarbital. No immuno-reactivity corresponding to cytochrome P450IIA was present in female F344 rats.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Enzyme and antibody inhibition/induction studies demonstrate that constitutively-expressed hepatic microsomal cytochrome P450IIA and P450IIB are the isozymes primarily responsible for conversion of 4-VCH to the 1,2 epoxide by untreated female B6C3F1 mice. Constitutive forms of cytochrome P450IIB present in female F344 rat liver are also able to metabolise 4-VCH to the epoxide, however this was a relatively minor pathway in control rats relative to that present in control mice. These differences in enzyme expression and metabolism of 4-VCH may be responsible for the differential susceptibility of rats and mice to 4VCH-induced ovarian neoplasia.

Reliability: (2) valid with restrictions
Study available for review. Non-guideline experimental study. Reasonably well reported methods and results, acceptable for evaluation.

10-JUL-2006 (59)

In Vitro/in vivo: In vitro
Type: Metabolism
Species: rat

Method: Washed hepatic microsomal preparations from untreated male Wistar rats (180-200 g) were used to investigate the NADPH-dependent metabolism of 4-VCH to monoepoxide and diol

products in vitro (pH 7.4, 37 degrees C, 5 min). The incubation mixtures were extracted with n-hexane (d-limonene as internal standard for epoxy metabolites) or ethyl acetate (n-tetradec-1-ene internal standard for diol metabolites), and quantified by GC-FID with structure confirmed by MS.

Result: Incubation of 4-VCH with rat microsomal fraction and a NADPH regenerating system resulted in the formation of 4-vinylcyclohex-1-ene 1,2-glycol (4-VCH 1,2 DL) and (-(1',2'-dihydroxyethyl)-cyclohex-1-ene (4-VCH 7,8 DL) in the ratio 3.5:1. Inclusion of 3,3,3-trichloropropene oxide (TCPO) in the incubation mixture lead to formation of the 1,2-epoxide (4-VCH 1,2 EP) and the 1',2' epoxide (4-VCH 7,8 EP) in the ratio 4:1, with complete inhibition of diol formation.

	------ pmol/mg protein/min ------			
	1,2 EP	7,8 EP	1,2 DL	7,8 DL
- TCPO	ND	ND	534	150
+ TCPO	494	120	ND	ND

ND = not detected

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Results from this study demonstrate that metabolism 4-VCH by rat hepatic microsomal enzymes to monoepoxide and diol products, with epoxidation occurring preferentially at the C1-double bond. Detection of the 1,2 and 7,8 monoepoxide products in vitro is only possible, however, after inclusion the epoxide hydrolase inhibitor 3,3,3-trichloropropene, suggesting further metabolism to the diol is normally rapid.

Reliability: (2) valid with restrictions
Study available for review. Non-guideline experimental study. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (68)

In Vitro/in vivo: In vitro
Type: Metabolism
Species: mouse

Method: Male albino Swiss mice (25-35 g; n = 5-8 per treatment) were given 4-VCH, 4-vinylcyclohexene monoxide (4-VCH MO; isomeric form not stated) and 4-vinylcyclohexene dioxide (4-VCH DO; isomeric form not stated) by i.p. injection (500 mg/kg body weight/day in corn oil, on two consecutive days; 0.3-0.5 ml corn oil per injection).

Animals were sacrificed 24 hr after the second injection, the livers removed and pooled cytosol- and microsomal fractions isolated by differential centrifugation.

Cytochrome P450 and b5 content, NADPH cytochrome c reductase activity, aminopyrine-N-demethylase activity, p-nitroanisole-O-demethylase activity, glutathione-S-transferase activity (toward styrene oxide) and epoxide hydrolase activity (toward safrole oxide) were

quantified using standard methods (3-4 replicates per assay).

Kinetic constants (Km, Vmax) for the interaction of 4-VCH DO with mouse hepatic glutathione-S-transferase was also investigated (no further details).

The impact of 4-VCH and its monoxide and dioxide metabolites on cytosolic glutathione concentration was also investigated (time-course study with animals sacrificed 0, 1, 2, 4, 10 and 24 hr post-dose).

Differences between the groups were analyzed using Student's t-test.

Result: Aminopyrine-N-demethylase (AP-N-D), NADPH-cytochrome c reductase (Cyt c) and epoxide hydrolase (EH) activities and microsomal content cytochrome P450 (P450) content were increased significantly in mice treated with 4-VCH and 4-VCH monoxide:

	nmol/min/mg protein			nmol/mg protein
	AP-N-D	Cyt c	EH	P450
Control (corn oil)	12.5	51	105	0.86
4-VCH	21.5*	74*	113	0.96
4-VCH MO	28.7*	102*	147*	1.26*

Comment: p-Nitroanisole-O-demethylase, cytochrome b5 content and glutathione-S-transferase activity were comparable in control and treated animals and are not tabulated above.
Comment : comparable data for 4-VCH DO treated mice not collected/reported.

Graphical results indicated that the glutathione (GSH) content of mouse liver declined markedly 1-4 hr after treatment with 4-VCH and its monoxide- and dioxide metabolite, but had recovered to control levels within 24 hr post-dose:

	----- GSH content -----		
Time (hr)	4-VCH	4-VCH MO	4-VCH DO
0	100%	100%	100%
1	42%	13%	10%
2	19%	12%	4%
4	5%	10%	- (a)
10	62%	69%	45%
24	93%	98%	93%

(a) = data not reported
Values obtained by interpolation from graphical data.

A Km of 3.7 mM and Vmax of 66 nmol/min/mg protein were obtained for 4-VCH DO and mouse hepatic glutathione transferase.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Pre-treatment of male mice with 4-VCH or its monoxide (2 x 500 mg/kg body weight, i.p.) altered expression of certain hepatic microsomal enzymes (N-demethylase, cytochrome c reductase, epoxide hydrolase) and cytochrome P450 content, while a single

dose i.p. treatment with 4-VCH or its monoxide- or dioxide metabolites rapidly decreased hepatic glutathione levels within 1-2 hours of treatment. 4-VCH dioxide was also shown to be a good substrate for mouse hepatic glutathione transferase (Km 3.7 mM, Vmax 66 nmol/min/mg protein). These findings suggest that 4-VCH may modulate its own metabolism in vivo.

Reliability: (2) valid with restrictions
Study available for review. Non-guideline experimental study. Briefly reported methods, adequate results, suitable for evaluation.

10-JUL-2006 (23)

In Vitro/in vivo: In vitro
Type: Metabolism
Species: other: rats and mice

Method: Liver, lung and ovary microsomal fractions were prepared from female Crl:CD BR rats (approx. 42-71 days old, body weight 200-300g) and female B6C3F1 mice (approx. 72 days old, body weight 20-27 g) by differential centrifugation, and stored frozen at -80 degrees C until use.

Experimental incubations (15 min, 37 degrees C) were performed in sealed vials containing microsomal fraction in phosphate buffer (pH 7.4), magnesium chloride and EDTA. An NADPH regenerating was included in incubations where cytochrome P450-dependent metabolism was predicted but omitted when epoxide hydrolase activity (NADPH-independent process) was monitored. 1,1,1-Trichloropropene (inhibitor of epoxide hydrolase) was included in experiments where formation of an epoxide metabolite was predicted. Control incubations (containing boiled microsomal fraction) were run in parallel.

The following metabolic processes were investigated:
- conversion of 4-VCH to 4-VCH 1,2-epoxide and 4-VCH 7,8-epoxide;
- conversion of 4-VCH 1,2-epoxide to 4-VCH diepoxide and 4-VCH 1,2-diol;
- conversion of 4-VCH 7,8-epoxide to 4-VCH diepoxide and 4-VCH 7,8-diol;
- hydrolysis of 4-VCH diepoxide.

Initial concentrations for each assay (not reported) were stated to be in excess of the limit of detection for each substrate and covered at least one order of magnitude with an upper limit of 5 mM. With the exception of mouse ovary samples (limited tissue availability), incubations were performed in duplicate.

Samples were extracted with ice cold ethyl acetate after addition of cyclodecane internal standard and analyzed by GC-FID.

The air:microsomal fraction partition coefficient for 4-VCH was determined (Gargas et al (1989) Toxicol. Appl. Pharmacol. 98, 87-99) and used to correct for evaporative losses during the experimental incubations.

Rates of metabolism, corrected for evaporative loss, were calculated per nmol cytochrome P450 and/or per mg microsomal protein. Kinetic constants (Vmax, Km) were obtained using the EZ-FIT computer program.

Measurement of other parameters (microsomal cytochrome P450 content, microsomal protein) followed standard methods.

Result: Conversion of 4-VCH to 4-VCH 1,2-epoxide:
Metabolism of 4-VCH to the 1,2 epoxide proceeded at detectable rates in liver and lung (undetectable in ovary from either species) and returned the following kinetic constants:

	Km	Vmax	
	(mM)	per mg protein	per nmol P450
Rat liver	1.58	0.20	0.13
Mouse liver	2.71	11.1	7.36
Rat lung	1.06	1.39	7.64
Mouse lung	0.61	3.49	29.5

Comment: Vmax presented as nmol/min/mg microsomal protein and nmol/min/nmol cytochrome P450.

Vmax/Km ratios for formation of the 1,2 epoxide by liver and lung (when expressed per mg protein and per mg P450) was markedly greater for mice compared to rats:

	Vmax/Km	
	per mg protein	per nmol P450
Rat liver	0.13	0.08
Mouse liver	4.10	2.71
Rat lung	1.31	7.21
Mouse lung	5.72	48.4

Conversion of 4-VCH to 4-VCH 7,8-epoxide:
Metabolism of 4-VCH to the 7,8 epoxide proceeded at low but detectable rates in liver and mouse lung (undetectable in rat lung or ovary from either species) and returned the following kinetic constants:

	Km	Vmax	
	(mM)	per mg protein	per nmol P450
Rat liver	1.10	0.007	0.005
Mouse liver	2.14	0.91	0.61
Rat lung	ND	ND	ND
Mouse lung	0.67	1.83	15.5

ND = not detected
Comment: Vmax presented as nmol/min/mg microsomal protein and nmol/min/nmol cytochrome P450.

Vmax/Km ratios for formation of the 7,8 epoxide (when expressed per mg protein and per mg P450) were greater for mice tissue compared to rat tissue:

	Vmax/Km	

	per mg protein	per nmol P450
Rat liver	0.006	0.005
Mouse liver	0.43	0.29
Rat lung	--	--
Mouse lung	2.73	23.1

Conversion of 4-VCH 1,2-epoxide to 4-VCH 1,2 diepoxide: Metabolism of the 1,2 epoxide to 4-VCH diepoxide was detectable in liver and lung from rats and mice (undetectable in ovary from either species) and returned the following kinetic constants:

	Km	Vmax	
	(mM)	per mg protein	per nmol P450
Rat liver	0.59	3.69	3.80
Mouse liver	0.51	5.35	3.64
Rat lung	0.29	2.06	14.4
Mouse lung	0.10	2.70	12.7

Comment: Vmax presented as nmol/min/mg microsomal protein and nmol/min/nmol cytochrome P450.

Vmax/Km ratios for formation of the diepoxide (when expressed per mg protein and per mg P450) were greater for mice tissue compared to rat tissue:

	Vmax/Km	
	per mg protein	per nmol P450
Rat liver	6.25	6.44
Mouse liver	10.5	7.13
Rat lung	7.10	49.7
Mouse lung	27.0	127

Conversion of 4-VCH 7,8-epoxide to 4-VCH diepoxide: Metabolism of the 7,8 epoxide to 4-VCH diepoxide was detectable in liver and lung from rats and mice (undetectable in ovary from either species) and returned the following kinetic constants:

	Km	Vmax	
	(mM)	per mg protein	per nmol P450
Rat liver	0.67	9.45	6.84
Mouse liver	0.57	8.83	5.94
Rat lung	0.60	1.35	11.2
Mouse lung	0.20	11.8	59.8

Comment: Vmax presented as nmol/min/mg microsomal protein and nmol/min/nmol cytochrome P450.

Vmax/Km ratios for formation of the diepoxide from the 7,8 epoxide (when expressed per mg protein and per mg P450) were comparable in liver but greater for mouse lung when compared to rat lung:

	Vmax/Km	
	per mg protein	per nmol P450
Rat liver	14.1	10.2
Mouse liver	15.5	10.2
Rat lung	2.25	18.7

Mouse lung	59.0	299

Conversion of 4-VCH epoxides to 4-VCH diols:
Metabolism of the 1,2 epoxide to 4-VCH 1,2 diol occurred only in liver (both species), and conversion of the 7,8 epoxide to 4-VCH 7,8 diol only in rat liver:

	1,2 epoxide		7,8 epoxide	
	Km (mM)	Vmax(+)	Km (mM)	Vmax(+)
Rat liver	0.19	6.53	0.57	135.8
Mouse liver	0.14	5.76	ND	ND

ND = not detected
(+) = expressed only per mg microsomal protein (independent of cytochrome P450)

Vmax/Km ratios for formation of the 1,2 diol from the 1,2 epoxide were 34.4 and 41.1 for rat and mouse liver, respectively, and 238 for formation of diol from the 7,8 epoxide by rat liver.

Hydrolysis of 4-VCH diepoxide:
Hydrolysis of 4-VCH diepoxide (presumed to a tetrol metabolite) was detectable in rat and mouse liver and lung, and in rat ovary:

	Km (mM)	Vmax(+)	Vmax/Km
Rat liver	0.19	5.51	29.0
Mouse liver	0.03	0.63	21.0
Rat lung	--(a)	0.39	
Mouse lung	--	1.06	
Rat ovary	--	0.90	
Mouse ovary	--(b)		

(+) = expressed only per mg microsomal protein (independent of cytochrome P450)
(a) = insufficient data for calculation
(b) = insufficient tissue to perform experiment

Vmax/Km ratios for hydrolysis of the diepoxide were comparable for rat and mouse liver.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Metabolic processes leading to the formation of 4-VCH epoxides and diepoxides were generally more active (higher Vmax, lower Km) 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 also in rat ovary microsomes (insufficient material for studies on mouse ovary), with the greatest Vmax returned by rat liver. Species differences in the balance of these activation and detoxication processes may result in lower systemic exposure to epoxide- and diepoxide metabolites in the rat relative to the mouse after equivalent exposures to 4-VCH.

Reliability: (2) valid with restrictions
Study available for review. Non-guideline experimental study.

Well reported methods and results, acceptable for evaluation.
10-JUL-2006 (33)

In Vitro/in vivo: In vitro
Type: Metabolism
Species: human

Method: Samples of human liver were obtained from 2 sources. Eight samples were obtained from organ donors accidents victims. Four samples were obtained from patients undergoing liver tumor removal, with normal liver tissue resected away from tumorous material.

All samples were placed in Sack's buffer for not more than 6 hours before microsome preparation. The microsomal fraction was isolated by differential centrifugation, and metabolic functionality determined by measuring cytochrome c reductase activity, cytochrome P450 content, and aniline hydroxylase activity.

The methods used for microsomal incubation and 4-VCH epoxidation were reported as in Smith, et al (1990a). Vials containing microsomal protein, NADP, glucose-6-phosphate dehydrogenase, glucose-6-phosphate. MgCl2, EDTA, 4-VCH, and 3,3,3-trichloroprene oxide in methanol, were placed in Hepe's buffer. Samples were pre-incubated for 3 min at 37 degrees C, The reaction was initiated by the addition of glucose-6-phosphate. The reaction was terminated with 0.2 vol of 5M sodium hydroxide. After organic extraction, 4-VCH-epoxide produced was analyzed by gas-liquid chromatography.

Result: Human microsomes, from 12 human livers, metabolized 4-VCH,in vitro, to VCH-1,2- or 7,8-epoxides, even in the absence of glucose-6-phospate. The major metabolite was VCH-1,2-epoxide. The rates of production of the 1,2-epoxide ranged from 0.23 to 1.25 nmol/mg microsomal protein/min. VCH-7,8-epoxide was formed at rates approximately 6 fold slower than the 1,2-epoxide.

Sample	Sex	VCH-1,2-Epoxide (nmol/min/mg)	VCH-7,8-Epoxide (nmol/min/mg)
D08	M	0.23	<0.01
D09	M	0.68	0.11
D10	M	0.85	0.11
D14	M	0.53	<0.01
D13	M	0.56	0.08
D14	M	0.54	<0.01
D07	F	0.45	0.06
D20	F	0.36	0.07
R09	F	0.82	0.15
R10	F	1.14	0.20

R12	F	0.68	0.11
R13	F	1.25	0.21

No dramatic differences were observed between males and females in the production of VCH-1,2-epoxide:

Parameter	Male/Female	Female	Male
nmol/min/mg	0.67±0.30	0.71±0.35	0.57±0.20
Number of samples	12	5	6

Rates of microsomal epoxidation of 4-VCH were mouse>rat>human (when plotted against rates of mouse and rat epoxidation from a previous paper).

The addition of an epoxide hydrolase inhibitor was required to detect the presence of VCH epoxides in human microsomes.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: The results demonstrate the presence of detectable levels of 4-VCH epoxidase activity in human human hepatic microsomal fractions in vitro.

Reliability: (2) valid with restrictions
Study available for review. Non-guideline experimental study. Reasonably well reported methods and results, acceptable for evaluation.

10-JUL-2006 (56)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: other: Carworth-Wistar
Sex: male/female
No. of Animals: 5
Doses: log 2 series
Value: = 3.08 ml/kg bw

Method: other: see methods
GLP: no

Method: Groups of 5 rats (age 4-5 weeks, bw 90-120 g) were intubated and given a single dose of undiluted 4-VCH (doses arranged in a log 2 series). Rats observed for 14 days. LD50 calculated by the method of Thompson.
Remark: This LD50 value has been referenced many times in the literature, however review of the primary data source reveals that this information is for screening purposes only, thus the methods used are not well documented.
Result: LD50 = 3.08 ml/kg bw (+/- 1.96 SD = 2.49-3.81 ml/kg bw) after oral gavage administration.

Comment: based on a density of 0.8299 g/ml, this is equivalent to 2560 mg/kg body weight.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
Study available for review. Pre-guideline, pre-GLP investigation. Briefly described methods, limited reporting of results but acceptable for assessment.
10-JUL-2006 (60) (61)

5.1.2 Acute Inhalation Toxicity

Type: LC0
Species: rat
Strain: no data
Sex: male/female
No. of Animals: 6
Doses: limit test: saturated vapor

Method: other: see methods
GLP: no

Method: 6 male or female albino rats were exposed to vapor-laden air for exposure periods of 15 min to 8 hr (log 2 series).
Remark: This value has been referenced many times in the literature, however review of the primary data source reveals that this information is for screening purposes only, thus the methods

used are not well documented.
Result: THere were no deaths following 15 minute exposure to a saturated atmosphere of 4-VCH vapor.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
Study available for review. Pre-guideline, pre-GLP investigation. Briefly described methods, limited reporting of results but acceptable for assessment.
10-JUL-2006 (60) (61)

Type: LC50
Species: rat
Strain: no data
Sex: male/female
No. of Animals: 6
Doses: limit test: 8000 ppm
Value: < 8000 ppm

Method: other: see methods
GLP: no

Method: Groups of 6 male or female albino rats were exposed to 8000 ppm 4-VCH vapor for 4 hr, then observed for a 14 day follow-up period. The reported exposure was a nominal value (based on weight of material vaporized) and not verified analytically.
Remark: This LC50 value has been referenced many times in the literature, however review of the primary data source reveals that this information is for screening purposes only, thus the methods used are not well documented.
Result: A 4 hr exposure of 8000 ppm 4-VCH killed 4/6 rats, indicating the LC50 is below 8000 ppm.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
Study available for review. Pre-guideline, pre-GLP investigation. Briefly described methods, limited reporting of results but acceptable for assessment.
10-JUL-2006 (60) (61)

Remark: A LC50 value of 6095 ppm is reported for the rat.

This toxicity value has been referenced by others in the literature; however the primary data source has not been discovered. This report is a review document, from 2001.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (4) not assignable
Secondary literature.
10-JUL-2006 (1)

Remark: A LC50 value of 10610 ppm is reported for the mouse.

This toxicity value has been referenced by others in the literature; however the primary data source has not been discovered. This report is a review document, from 2001.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (4) not assignable
10-JUL-2006 (1)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain: New Zealand white
Sex: male
No. of Animals: 4
Doses: not reported
Value: >= 20 ml/kg bw

Method: other: see methods
GLP: no

Method: Fur was clipped from the trunk of 4 male rabbits. Dose applied and occluded with an impervious plastic film for 24 hours, during which time animals were immobilized. Observed for 14 days post-treatment. LD50 measured using the Thompson method.
Remark: This LD50 value has been referenced many times in the literature, however review of the primary data source reveals that this information is for screening purposes only, thus the methods used are not well documented.
Result: LD50 = 20 ml/kg bw, 24 hr occluded application.

Comment: the method notes that treatment volumes in excess of 20 ml/kg cannot be retained in contact with the skin. It is therefore possible that this was a 'limit test' and that the actual LD50 was, in fact, greater than 20 ml/kg. No information on mortality was provided, however.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Reliability: (2) valid with restrictions
Study available for review. Pre-guideline, pre-GLP investigation. Briefly described methods, limited reporting of results but acceptable for assessment.
10-JUL-2006 (60) (61)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration: other: undiluted
Exposure: Open
Exposure Time: 24 hour(s)
No. of Animals: 5
Result: moderately irritating

Method: other: see methods
GLP: no

Method: Skin reactions were recorded 24 hr after application of 0.01 ml of undiluted sample to clipped albino rabbit skin (n = 5 animals).

Results are based on the severest reaction present, based on the following scale:
Grade 1 = no reaction
Grade 2 = minimal capillary injection
Grade 6 = necrosis

Comment: the test site was uncovered (non-occluded) and rapid evaporative loss of test sample seems probable.

Remark: This irritation value has been referenced many times in the literature, however review of the primary data source reveals that this information is for screening purposes only, thus the methods used are not well documented.

Result: Moderate skin irritation (Grade 4) was reported using the authors' own scoring system.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Reliability: (2) valid with restrictions
Study available for review. Pre-guideline, pre-GLP investigation. Briefly described methods, limited reporting of results but acceptable for assessment.

10-JUL-2006 (60) (61)

5.2.2 Eye Irritation

Species: rabbit
Concentration: other: undiluted
Dose: other: not stated
Exposure Time: unspecified
Result: slightly irritating

Method: other: see methods
GLP: no

Method: Corneal reactions were recorded following instillation into rabbit eye.

Results represent the degree of corneal necrosis present, based on the following scale:

Grade 1 = very small area affected, resulting from instillation of 0.5 ml undiluted substance

Grade 5 = severe burn following instillation of 0.005 ml undiluted test substance.

Comment: group sizes not reported.

Remark: This irritation value has been referenced many times in the literature, however review of the primary data source reveals that this information is for screening purposes only, thus the methods used are not well documented.

Result: Minimal corneal irritation (Grade 2) was reported using the authors' own scoring system.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Reliability: (2) valid with restrictions
Study available for review. Pre-guideline, pre-GLP investigation. Briefly described methods, limited reporting of results but acceptable for assessment.

10-JUL-2006 (60) (61)

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 2 wk
Frequency of treatment: 6 hr/d, 5 d/wk; 1 rest day between 1st and 2nd wk
Post exposure period: 3 d
Doses: 0, 240, 720 or 1500 ppm
Control Group: yes, concurrent vehicle
NOAEL: = 720 - 1500 ppm

Method: EPA OTS 798.2450
GLP: yes

Method: Male and female Sprague-Dawley rats (5/sex/dose level) were exposed by inhalation to 4-VCH (0 (air), 240, 720 or 1500 ppm) 6 hr/day, 5 days/week for 2 weeks, with 1 day of rest between each week.

Animals individually housed in stainless steel cages, with free access to Purina Rodent Chow no. 5002 and tap water during non-exposure periods. Observed and weighed daily and monitored during and after exposure period for clinical signs.

The achieved concentration within each chamber was monitored (GC-FID) approximately once every 30 min during each 6 hr exposure.

Statistical analysis was conducted on body weights.

Result: Mean body weight gain over study days 1-11 was significantly decreased in high dose males relative to controls, and numerically (but not significantly) decreased in high dose females. Final body weights were also were decreased non-significantly decreased in these animals:

Body weight, day 11:
- males 100%, 97%, 95%, 89%
- females 100%, 99%, 98%, 96%

Body weight gain, days 1-11:
- males 100%, 97%, 91%, 72% *
- females 100%, 98%, 92%, 87%

* P <0.05

A single mid-dose female was found dead on study day 2 (presumed unrelated to treatment), and replaced. All other animals survived to the end of the recovery period.

Reversible lethargy was noted in all rats from the mid- and high dose groups following removal from the exposure chambers. Tremor (affecting 1/10 low dose rats and 3/10 high dose rats) was observed on study day 3 only (absent on other occasions).

Based on body weight gain over study days 1-11, a NOAEC of 720 ppm was derived for male rats and 1500 ppm for females.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Decreased body weight was the principal finding in rats exposed to 4-VCH by inhalation, with a NOAEC of 720 ppm for males and 1500 ppm (the highest dose tested) for females.

Reliability: (1) valid without restriction
Study available for review. Comparable to guideline study. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (19)

Type: Sub-acute
Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of administration: inhalation
Exposure period: 2 wk
Frequency of treatment: 6 hr/d, 5 d/wk; 1 rest day between 1st and 2nd wk
Post exposure period: 3 d
Doses: 0, 240, 720 or 1500 ppm
Control Group: yes, concurrent vehicle
NOAEL: = 720 ppm

Method: EPA OTS 798.2450
GLP: yes

Method: Male and female B6C3F1 mice were exposed by inhalation to 4-VCH (0 (air), 240, 720 or 1500 ppm) 6 hr/day, 5 days/week for 2 weeks, with 1 day of rest between each week.

[Other methodological details as for the rat 2 wk inhalation study, described elsewhere in this section.]

Result: All groups of mice, including controls, lost weight over study days 1-3. This effect was particularly marked in high dose animals (both sexes) which lost 18-20% of their initial body weight (statistically significant) during this time.

All high dose males, and 4/5 high dose females, were found dead on study day 4. (Remaining high dose female sacrificed in extremis on study day 4.)

Mice from the control, low and mid dose groups exhibited inconsistent increases in body weight over the remainder of the study (i.e. from study day onwards):

Body weight gain, males:
- days 1-11: 1.7g 1.5g 1.6g
- days 11-14 -1.0g -0.3g -0.2g

Body weight gain, females:
- days 1-11: 2.0g 0.0g* 1.5g
- days 11-14 -1.1g 0.7g* -1.0g

* P <0.05

Reversible lethargy was seen in all mice from the mid- and high dose groups after removal from the exposure chambers. Tremor was present in 7/10 mice on study day 3, and was considered by the report as a significant feature preceeding death.

Based on tremor and mortality recorded after exposure to 1500 ppm 4-VCH, a NOAEC of 720 ppm was obtained for male and female mice.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Conclusion: Tremor and mortality were the principal finding in mice

exposed to 4-VCH by inhalation, with a NOAEC of 720 ppm for males and females.

Reliability: (1) valid without restriction
Study available for review. Comparable to guideline study. Well reported methods and results, acceptable for evaluation.
10-JUL-2006 (19)

Type: Sub-acute
Species: rat **Sex:** male/female
Strain: Fischer 344
Route of administration: gavage
Exposure period: 2 wk
Frequency of treatment: consecutive days
Post exposure period: none
Doses: 0, 300, 600, 1250, 2500 or 5000 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL: = 600 mg/kg bw
LOAEL: = 1250 mg/kg

Method: other: standard NTP methodology
GLP: no data

Method: Groups of 5 male and 5 female F344 rats (Charles River Breeding Laboratories; age 7 wk at start of treatment) were administered 4-VCH (>99% pure) in corn oil by gavage at doses of 0, 300,600, 1250, 2500 or 5000 mg/kg bw/d for 14 consecutive days. Dose volume = 5.81 ml/kg.

The animals were group housed (5/sex/cage) with feed (Lab Chow Checkers) and tap water (acidified to pH 2.5 to prevent bacterial growth) ad libitum, and observed twice daily for mortality and once daily for clinical signs. Body weight recorded on day 0 and day 14.

Necropsies were performed on all animals (macroscopic observations only, histopathology limited to stomach = putative target organ).

Dosing solutions prepared at least weekly (stored at room temperature), achieved concentration and stability determined using GC-FID.

It is not stated if any statistical analysis was applied to the data.

Result: GC-FID demonstrated that the achieved concentration of dosing solutions was +/- 10% of nominal.

All rats given 1250 mg/kg bw/d and above died before the end of the study. Moribund animals were inactive with perianal wetness, tremors, soft stools and an unsteady gait. There were no substance-related deaths at lower doses.

Mean weight gain among survivors (including controls) over the 14 d of the study was highly erratic:

- males: -53g, +10 g, -4 g
- females: +1 g, -1 g, -3 g
(Results by dose level for 0, 300 and 600 mg/kg bw/d groups)

No gross lesions were detected at necropsy, or in the stomach following microscopic evaluation.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of the study, the sub-acute NOAEL for 4-VCH in male and female rats was 600 mg/kg bw/d (based on survival).

Reliability: (1) valid without restriction
Study available for review. Comparable to guideline study. Briefly reported methods and results, acceptable for evaluation.

10-JUL-2006 (14) (50)

Type: Sub-acute
Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of administration: gavage
Exposure period: 2 wk
Frequency of treatment: consecutive days
Post exposure period: none
Doses: 0, 300, 600, 1250, 2500 or 5000 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL: = 1250 mg/kg bw
LOAEL: = 2500 mg/kg bw

Method: other: standard NTP methodology
GLP: no data

Method: Groups of 5 male and 5 female B6C3F1 mice (Charles River Breeding Laboratories; age 8 wk at start of treatment) were administered 4-VCH (>99% pure) in corn oil by gavage at doses of 0, 300, 600, 1250, 2500 or 5000 mg/kg bw/d for 14 consecutive days. Dose volume = 5.81 ml/kg.

[Other methodological details as reported above for the rat 14 d sub-acute study.]

Result: GC-FID demonstrated that the achieved concentration of dosing solutions was +/- 10% of nominal.

All mice given 2500 mg/kg bw/d and above, and 3/5 males given 1250 mg/kg bw/d, died before the end of the study. Moribund animals were inactive with tremors. There were no substance-related deaths at lower doses.

With the exception of females given 300 mg/kg bw/d, all groups of survivors (including controls) lost weight over the 14 d of the study:
- males: -1.6 g, -1.6 g, -1.8 g, -2.0 g
- females: -1.4 g, +0.6 g, -1.4 g, -1.4 g
(Results by dose level for 0, 300, 600 and 1250 mg/kg bw/d groups)

No gross lesions were detected at necropsy, or in the stomach following microscopic evaluation.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of the study, the sub-acute NOAEL for 4-VCH in male and female mice was 1250 mg/kg bw/d (based on survival).

Reliability: (1) valid without restriction
Study available for review. Comparable to guideline study. Briefly reported methods and results, acceptable for evaluation.

10-JUL-2006 (14) (50)

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 13 wk
Frequency of treatment: 6 hr/d, 5 d/wk
Post exposure period: none
Doses: 0, 250, 1000 or 1500 ppm
Control Group: yes, concurrent vehicle
NOAEL: = 250 ppm
LOAEL: = 1000 ppm

Method: EPA OTS 798.2450
GLP: yes

Method: Male and female Sprague-Dawley rats were exposed by inhalation to 4-VCH (0 (air), 250, 1000 or 1500 ppm) 6 hr/day, 5 days/week for 13 weeks. In addition, another group of rats was exposed to 1000 ppm butadiene to permit comparison between the two compounds.

Animals individually housed in stainless steel cages. Free access to Purina Rodent Chow no. 5002 and tap water during non-exposure periods.

The achieved concentration in the chambers was monitored (GC-FID) approximately once every 30 min during each 6 hr exposure.

Animals observed daily during and after exposure period for clinical signs. Observations were made twice daily for morbundity and mortality on weekdays and once daily on weekends. Body weights were recorded weekly and food consumption was determined.

Hematological and serum chemistry, as well as urine analysis, were performed on all animals surviving to study termination.

Necropsies were performed on all decedent and surviving animals, and a comprehensive range of tissues from the controls and 1500 ppm group were subject to microscopic examination.

Comprehensive statistical analysis was conducted on body weights, body weight gains, organ weights, and clinical laboratory measurements.

Result: The most notable compound-related clinical sign was lethargy observed in rats exposed to 1500 ppm 4-VCH.

Male rats exposed to 1500 ppm 4-VCH had significantly lower body weights compared to controls, with significantly lower body weight gains in both sexes at this level.

None of the 4-VCH-exposed (butadiene-exposed) rats showed any compound-related alteration in hematological, clinical chemistry or urine parameters.

Absolute and/or relative liver weights were increased in both sexes exposed to 1000 or 1500 ppm 4-VCH or 1000 ppm butadiene, with increased renal weights in these males. Microscopically, increased accumulation of hyaline droplets was observed in the kidneys of male rats from all 4-VCH exposure groups. Although compound-related, the droplets were not accompanied by cytotoxicity.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: For 4-VCH exposure, the no-observed-adverse-effect-level is 250 ppm for rats based on organ weight increases at higher exposures.

Reliability: (1) valid without restriction
Study available for review. Comparable to guideline study. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (6)

Type: Sub-chronic
Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of administration: inhalation
Exposure period: 13 wk
Frequency of treatment: 6 hr/d, 5 d/wk
Post exposure period: none
Doses: 0, 50, 250 or 1000 ppm
Control Group: yes, concurrent vehicle
NOAEL: = 250 ppm
LOAEL: = 1000 ppm

Method: EPA OTS 798.2450
GLP: yes

Method: Male and female B6C3F1 mice were exposed by inhalation to 4-VCH (0 (air), 50, 250 or 1000 ppm) 6 hr/day, 5 days/week for 13 weeks. In addition, another group of mice was exposed to 1000 ppm butadiene to permit comparison between the two compounds.

[With the exception of urinanalysis (not conducted on mice), other methodological details as for the rat 13 wk inhalation study described elsewhere in this section.]

Result: Exposure to 1000 ppm 4-VCH resulted in deaths of all male mice and 5/10 female mice on test days 11 or 12. Three additional female mice exposed to 1000 ppm VCH died prior to study completion.

The most notable compound-related clinical sign was lethargy observed in the 1000 ppm 4-VCH-exposed mice.

None of the 4-VCH-exposed animals showed any compound-related hematological effects, although mild macrocytic anemia was present in positive control mice exposed to 1000 ppm butadiene.

The most notable histopathological finding was ovarian atrophy in females exposed to 1000 ppm 4-VCH or 1000 ppm butadiene (slightly more severe after 4-VCH-exposure than in the butadiene-exposed females). No other compound-related pathological effects in male or female mice exposed to 4-VCH.

Comment: butadiene-exposed male mice also had decreased testicular weights, accompanied by slight testicular degeneration and atrophy.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: For 4-VCH exposure, the no-observed-adverse-effect-level is 250 ppm for mice based on mortality and ovarian atrophy.

Reliability: (1) valid without restriction
Study available for review. Comparable to guideline study. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (6)

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: Fischer 344
Route of administration: gavage
Exposure period: 13 wk
Frequency of treatment: 5 d/wk
Post exposure period: none
Doses: 0, 50, 100, 200, 400 or 800 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL: = 200 - 400 mg/kg bw
LOAEL: = 400 - 800 mg/kg bw

Method: other: standard NTP methodology
GLP: no data

Method: Groups of 10 male and 10 female F344 rats (Charles River Breeding Laboratories; age 7 wk at start of treatment) were administered 4-VCH (>99% pure) in corn oil by gavage at doses of 0, 50, 100, 200 400 or 800 mg/kg bw/d 5 d/wk for 13 wk. Dose volume = 3.33 ml/kg.

The animals were group housed with feed (NIH 07 Rat and Mouse Ration pellets) and water (acidified to pH 2.5 to prevent bacterial growth) available ad libitum. They were observed

twice daily for mortality, and animals judged to be moribund taken to necropsy. Body weight and detailed clinical observations were recorded once per week.

Necropsies were performed on all animals surviving to the end of the treatment period. A comprehensive range of tissues (including blood smear) from the controls and 800 mg/kg bw/d group were subject to microscopic examination, together with the stomach (both sexes; putative target organ) and kidneys (males only) from the intermediate treatment groups.

Dosing solutions were prepared at least weekly (stored at room temperature), achieved concentration and stability determined using GC-FID.

It is not stated if any statistical analysis was applied to the data.

Result: GC analysis of the dosing solutions demonstrated that the achieved concentration was 95 - 100% of target.

There were premature deaths in single animals from the 400 mg/kg bw/d (male) and 800 mg/kg bw/d (female) groups. (No further details.)

Body weight gain was decreased in the higher dose males, with a less marked effect in females. Final bw by dose level relative to controls:
- males: 100%, 101%, 96%, 97%, 93%, 87%
- females: 100%, 101%, 96%, 97%, 99%, 94%

No gross macroscopic lesions are described in the report.

Microscopic examination revealed hyaline droplet degeneration of the proximal convoluted tubule of the kidney in males (not females). Severity was diagnosed as minimal for all groups with the exception of 800 mg/kg males (no further details; presumed mild). Inflammation of the submucosa of the nonglandular stomach (severity not defined) was present in 1 male and 3 females given 800 mg/kg bw/d. No other treatment-related histologic abnormalities present.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of the study, the sub-chronic NOAEL for 4-VCH in the rat was 200 mg/kg bw/d in males and 400 mg/kg bw/d in females (based on inflammation of the stomach and decreased terminal body weight at higher doses in both sexes).

Reliability: (1) valid without restriction
Study available for review. Comparable to guideline study. Briefly reported methods and results, acceptable for evaluation.

10-JUL-2006 (14) (50)

Type: Sub-chronic
Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of administration: gavage
Exposure period: 13 wk
Frequency of treatment: 5 d/wk
Post exposure period: none
Doses: 0, 75, 150, 300, 600 or 1200 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL: = 600 mg/kg bw
LOAEL: = 1200 mg/kg bw

Method: other: standard NTP methodology
GLP: no data

Method: Groups of 10 male and 10 female B6C3F1 mice (Charles River Breeding Laboratories; age 8 wk at start of treatment) were administered 4-VCH (>99% pure) in corn oil by gavage at doses of 0, 75, 150, 300, 600 or 1200 mg/kg bw/d 5 d/wk for 13 wk.

[Other methodological details as reported above for the rat 13 wk sub-chronic study.]

Result: GC analysis of the dosing solutions demonstrated that the achieved concentration was 95 - 100% of target.

A high level of early mortality was recorded for high dose males (9/10 dying in study wk 1-9) and high dose females (4/10, study wk 9 and 12), with lower mortality (2/10, study wk 12) in females given 300 mg/kg bw/d. (Other deaths (1 or 2 per group) for females from the 150-600 mg/kg bw/d groups were considered due to dosing errors; diagnosis based on tissue damage visible at necropsy).

Female mice from the 600 mg/kg bw/d groups weighed approx. 5% less than the corresponding controls, while body weight for the sole surviving high dose male was around 7% lower than the male controls. Body weights for the other groups of treated mice (including high dose females) were highly comparable to the controls.

Mild acute inflammation of the stomach was detected microscopically in 3 decedent males and one surviving female given 1200 mg/kg bw/d. Histological re-evaluation of ovaries from high dose females (decedents and survivors) revealed a decrease in the number of primary follicles and mature graafian follicles (no quantitative information provided; lower treatment groups not examined). No other lesions were present.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of the study, the sub-chronic NOAEL for 4-VCH in the mouse was 600 mg/kg bw/d for males (based on early mortality and stomach lesions) and females (based on early mortality and microscopic changes in stomach). Given an absence of gross lesion, the limited evaluation of any

microscopic changes present in tissues from the intermediate dose group and a relatively high incidence of mis-dosing reported in the study as a whole, no conclusions can be drawn as to the toxicological relevance of decreased survival recorded for females given 300 mg/kg bw/d.

Reliability: (1) valid without restriction
Study available for review. Comparable to guideline study. Briefly reported methods and results, acceptable for evaluation.

10-JUL-2006 (14) (50)

Type: Chronic
Species: rat **Sex:** male/female
Strain: Fischer 344
Route of administration: gavage
Exposure period: 103 wk
Frequency of treatment: 5 d/wk
Post exposure period: none
Doses: 0, 200 or 400 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL: < 200 mg/kg bw
LOAEL: = 200 mg/kg bw

Method: other: standard NTP methodology
GLP: no data

Method: Groups of 50 male and 50 female F344 rats (Charles River Breeding Laboratories ; age 7 wk at start of treatment) were administered 4-VCH (>98% pure) in corn oil by gavage at doses of 0, 200 or 400 mg/kg bw/d 5 d/wk for 103 wk. Dose volume = 3.33 ml/kg.

The animals were group housed with feed (NIH 07 Rat and Mouse Ration pellets) and acidified water (pH 2.5) available ad libitum in an air conditioned environment (22-24 deg. C, 30-70% rel. humidity, 12 hr light cycle, 12-15 air changes/hr). They were observed twice daily for mortality, once weekly for clinical signs, and palpated once monthly. Body weights were initially recorded weekly (study wk 1-13) then monthly thereafter. Any animals judged to be moribund taken to necropsy.

Necropsies were performed on all animals (survivors and decedents), and the following tissues sampled for processing (H&E staining) and microscopic examination:
gross lesions and masses
adrenal glands
blood smear
brain
colon
esophagus
eyes
heart
kidneys

liver
lung and mainstem bronchi
mammary gland
mandibular and mesenteric lymph nodes
ovaries/uterus
pancreas
parathyroid glands
pituitary gland
prostate/testes
regional lymph nodes
salivary glands
small intestine
spinal cord
stomach
sternebrae (incl. marrow)
thymus
thyroid
trachea
urinary bladder

Dosing solutions were prepared at least weekly (stored at room temperature), achieved concentration and stability determined using GC-FID.

The probability of survival was estimated using the procedure of Kaplan and Meier, with dose-related effects analyzed by the methods of Cox and of Tarone. (Animals dying from non-natural causes or missing from the study were excluded.) Where differences were present, additional analysis was carried out to identify the time point at which differences became significant. The Fisher exact test was used to compare the incidence of non-tumor lesions in control and treated animals.

Result: GC-FID analysis demonstrated that approx. 94% of the dosing solutions were within specification during the study:

	Nominal concentration (mg/ml)	
	60.1	120.1
Mean (mg/ml)	59.3	117.5
SD	3.54	4.58
Coeff. Varn	6.0	3.9
Range (mg/ml)	49.7-66.2	110.0-126.9
No. analyzed	17	17

Body weight and clinical signs
High dose males exhibited a 5-14% reduction in bw relative to controls from study wk 72; reason for this late weight loss not known. Other bw values (low dose males, all females) similar to controls.
Comment: non-optimal randomization resulted in marked bw differences at time of allocation to groups:
- males: 100%, 93%, 109%
- females: 100%, 113%, 114%
(initial bw as percentage of control, by treatment group)
No clinical signs were described.

Survival
Survival of high dose males was significantly lower than that of controls from wk 5 (5/50 alive at wk 103; P<0.001), and significantly lower for low dose males from wk 88 (13/50 alive at wk 103; P<0.001). Overall survival of high dose females was also lower than controls (13/50 alive at wk 103; P<0.001), and decreased non-significantly in low dose females (28/50 alive at wk 103).
Comment: the authors comment that there is no explanation for the poor survival of the high dose males from wk 5 i.e. not replicated at this treatment level in sub-chronic study, no gross or microscopic lesions detected.

Non-tumor pathology
The incidence of epithelial hyperplasia of the forestomach was higher in treated animals, particularly for males from the 400 mg/kg bw/d groups. Incidence by dose level:
- males: 2%, 6%, 11%
- females: 0%, 4%, 4%

This late-appearing lesion was increased significantly (P<0.01) for males surviving beyond wk 93. Incidence by dose level:
- males: 3%, 14%, 36%

There was a dose-related decrease in incidence of cataracts in males, and a dose related increase for females.
Comment: the authors suggest this may reflect placement of cages within the animal room (cannot be verified, records unavailable).

No other non-tumor microscopic lesions were reported.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of the study, decreased survival and epithelial hyperplasia in forestomach were recorded in male and female rats administered 4-VCH by oral gavage for 103 wk. The results support a chronic LOAEL of 200 mg/kg bw/d, based on the occurrence of effects in the low dose group (more pronounced in males than females).

Reliability: (1) valid without restriction
Study available for review. Comparable to guideline study. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (50)

Type: Chronic
Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of administration: gavage
Exposure period: 103 wk
Frequency of treatment: 5 d/wk
Post exposure period: none
Doses: 0, 200 or 400 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL: < 200 mg/kg bw
LOAEL: = 200 mg/kg bw

Method: other: standard NTP methodology
GLP: no data

Method: Groups of 50 male and 50 female B6C3F1 mice (Charles River Breeding Laboratories ; age 8 wk at start of treatment) were administered 4-VCH (>98% pure) in corn oil by gavage at doses of 0, 200 or 400 mg/kg bw/d 5 d/wk for 103 wk. Dose volume = 3.33 ml/kg.

[Other methodological details as reported above for the rat 103 wk study. Gall bladder was included in the list of tissues collected at necropsy for subsequent histopathological assessment.]

Result: GC-FID analysis demonstrated that approx. 94% of the dosing solutions were within specification during the study. [See rat 103 wk study, above, for further details.]

Body weight and clinical signs
Mean body weight was 5-13% lower in high dose male mice relative to controls between study wk 8-76, but had fully recovered by wk 100. In high dose females, mean body weight was at least 5% lower than control values from study wk 20, with a 12% weight reduction apparent at the end of the study. Smaller fluctuations (5-7% decreases) were also apparent in the low dose groups during the mid-phase of the study but this had resolved by study termination.

No clinical signs were described.

Survival
Survival of high males was decreased significantly relative to controls from study wk 29, with only 7/50 animals alive at study termination ($P<0.001$). Survival of high dose females lower than controls after wk 32, with 17/50 alive at wk 103 ($P<0.001$). No gross or microscopic observations present to explain reduction in survival. Survival of the low dose groups was comparable to that of the controls (39/50 alive at termination).

Non-tumor pathology
Ulcers, mild inflammation and epithelial hyperplasia of the forestomach was observed in both sexes. Incidence by dose

level:
- males:
ulcer: 0%, 6%, 15%
inflammation: 0%, 14%, 35%
epithelial hyperplasia: 0%, 14%, 15%
- females:
ulcer: 0%, 0%, 9%
inflammation: 2%, 4%, 22%
epithelial hyperplasia: 2%, 6%, 9%

Tubular cell hyperplasia, granulosa cell hyperplasia and papillary hyperplasia of the ovary observed at increased incidence in female mice. Incidence by dose level:
- females:
tubular cell hyperplasia: 0%, 21%, 28%
granulosa cell hyperplasia: 0%, 10%, 2%
papillary hyperplasia: 0%, 0%, 4%
(Comment: tumor site - see section 5.7)

Congestion of the lung recorded at increased incidence in high dose mice. Incidence by dose level:
- males: 4%, 4%, 72%
- females: 0%, 2%, 40%
In the absence of statistical analysis, findings in low dose animals are considered to be of doubtful toxicological relevance.
(Comment: tumor site - see section 5.7)

Atrophy of the spleenic red pulp observed at increased incidence in high dose males only (22% versus 0% in controls; absent from all other groups).
(Comment: tumor site (lymphoma) - see section 5.7)

The incidence of histological abnormalities of the adrenal gland increased in treated female mice (males unaffected). Incidence by dose level:
- alteration of the adrenal cortex (subcapsular cell hyperplasia, Type B cells): 0%, 49%, 29%
- congestion of the adrenal gland: 0%, 0%, 17%
(Comment: tumor site - see section 5.7)

Hepatic centrilobular congestion increased in high dose males only (14% versus 0% in controls; absent from all other groups).

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of the study, decreased survival and histopathological tissue alterations in adrenal gland, forestomach, liver, lung and spleen were recorded in male and female mice administered 4-VCH by oral gavage for 103 wk. The results support a chronic LOAEL of 200 mg/kg bw/d in males (based the presence of ulcers, mild inflammation and epithelial hyperplasia in forestomach) and females (based on histological abnormalities of the adrenal gland and ovary).

Reliability: (1) valid without restriction

Study available for review. Comparable to guideline study.
Well reported methods and results, acceptable for evaluation.
10-JUL-2006 (15) (50)

5.5 Genetic Toxicity 'in Vitro'

Type: Bacterial reverse mutation assay
System of testing: Salmonella typhimurium TA100, TA104, TA1535, TA97, TA98
Concentration: 1-10000 ug/plate or 1-1666 ug/plate
Cytotoxic Concentration: 333 ug/plate (slight toxicity -S9 and +S9)
Metabolic activation: with and without
Result: negative

Method: other: US-NTP standard protocol
GLP: no data

Remark: Only limited information is available for this study which was conducted in the absence or presence of 10% or 30% rat or hamster S9 using a preincubation protocol.

Tests were run with an independent repeat.

DMSO was the vehicle control with (currently unspecified) positive controls for each strain.
Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Conclusion: Under the conditions of the test, no mutagenic activity was detected in 5 strains of Salmonella typhimurium (including TA100, TA104, TA1535, TA97, TA98) in the absence or presence of rat or hamster S9.
Reliability: (2) valid with restrictions
Comparable to guideline study. Data tables and briefly reported methods/results available for review, acceptable for evaluation.
10-JUL-2006 (51)

Type: Bacterial reverse mutation assay
System of testing: Salmonella typhimurium TA100, TA1535, TA1537, and TA98
Concentration: 1-1000 ug/plate
Cytotoxic Concentration: 1000 ug/plate (at 10% Rat S9)
Metabolic activation: with and without
Result: negative

Method: other: US-NTP standard protocol
GLP: no data

Remark: Only limited information is available for this study which was conducted in the absence or presence of 10% rat or hamster S9 using a preincubation protocol.

Tests were run with an independent repeat.

DMSO was the vehicle control with (currently unspecified) positive controls for each strain.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.
Conclusion: Under the conditions of the test, no mutagenic activity was detected in 4 strains of Salmonella typhimurium (including TA100, TA1535, TA1537, and TA98) in the absence or presence of rat or hamster S9.
Reliability: (2) valid with restrictions
Comparable to guideline study. Data tables and briefly reported methods/results available for review, acceptable for evaluation.
10-JUL-2006 (47)

Type: Mammalian cell gene mutation assay
System of testing: mouse lymphoma L5178Y TK+/- cells
Concentration: 20 to 150 ug/mL
Metabolic activation: with and without
Result: positive

Method: other: US-NTP standard protocol
GLP: no data

Method: Treated cultures contained 6 x 10e6 cells in 10 mL of medium, which included the S9 fraction in those experiments performed with metabolic activation. Incubation with the test chemical continued for 4 hours, at which time the medium plus chemical was removed and the cells were re-suspended in 20 mL of fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained.

After the 48-hour expression period, 3 x 106 cells were plated in medium and soft agar supplemented with TFT for selection of TFT-resistant cells (TK-/-) and in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37 C. in 5% CO2 for 10 to 12 days. At the end of incubation, colonies were counted with an automated counter. The test was initially performed without S9. If a clearly positive response was not obtained, the test was repeated using freshly prepared S9 from the livers of either Aroclor 1254-induced or non-induced male Fischer 344 rats.

Each exposure concentration was tested in triplicate, and the experiment was performed with an independent repeat.

Minimum validity criteria included acceptable cloning efficiencies and relative total growth, absence of test chemical precipitate and two or more acceptable cultures per dose set.

Data were evaluated statistically for trend and peak responses. Both responses had to be significant ($P < 0.05$) for a chemical to be considered capable of inducing TFT resistance; a single significant response led to a "questionable" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call.

Result: An average mutation frequency of 112 mutants/10^6 surviving colonies was reported after exposure to 60 ug/mL, 149 after exposure to 80 ug/mL, 108 after exposure to 100 ug/mL, and 148 after exposure to 120 ug/mL in one of the three trials with S9 activation.

Elevated mutation frequency were observed also in the 2 other trials but the increases were considered equivocal.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of the assay, 4-VCH was reported to be mutagenic in mouse lymphoma cells in the presence of S9 metabolic activation. However, from the data presented, it is not clear if there was a statistically significant dose-related increase in the mutant frequency, or if the increases observed at specific concentrations was reproducible and statistically significant.

Reliability: (2) valid with restrictions
Comparable to guideline study. Data tables and briefly reported methods/results available for review but only limited information provided concerning statistical basis for study conclusions. Acceptable for evaluation.

10-JUL-2006 (52)

Type: Sister chromatid exchange assay
System of testing: Chinese Hamster Ovary (CHO) Cells in vitro
Concentration: 5, 16.7, 50 and 166.7 ug/mL.
Cytotoxic Concentration: 166.7 (presumed from data)
Metabolic activation: with and without
Result: negative

Method: other: US-NTP standard protocol
GLP: no data

Method: In experiments performed without S9, Chinese Hamster Ovary (CHO) cells were incubated with the test chemical for 26 hours in supplemented McCoy's 5A medium, with BrdU added 2 hours after culture initiation. The medium was replaced (no test chemical but BrdU and colcemid present) after 26 hours incubation, then cells harvested 2 hours later for fixation and staining (Hoechst 33258 and Giemsa).

In studies with S9 present, cells were incubated with the test chemical + S9 in serum-free medium 2 hours, the medium replaced (serum and BrdU present but no test chemical) and incubation continued for an additional 26 hours; colcemid was added for the final 2 hours. Cells were then fixed and stained as above.

Slides were scored blind, with 50 second-division metaphase cells evaluated to determine SCE frequency per cell for each dose level. If significant chemical-induced cell cycle delay was seen in treated cultures, the incubation time was lengthened to ensure the accumulation of a sufficient number of scorable (second-division metaphase) cells. Approximately

1020 chromosomes were examined at each dose.

Mitomycin C used as a positive control for tests performed without S9 activation, cyclophosphamide as a positive control in the presence of S9.

Statistical analyses were conducted to assess the presence of a dose-response (trend test) and the significance of the individual dose points was also compared to the vehicle control. A 20% increase in SCE frequency at any single dose was considered indicative of a weak positive response; increases at two or more doses indicated a positive result.

Result: The total number of SCE, SCE per chromosome, and SCE per cell were elevated approximately 8% and 12% over the solvent control at the 5 ug/mL and 50 ug/mL doses, and approximately 6% at the 16.7 ug/mL dose, with and without S9 activation.

Cells at the 166.7 ug/mL were not examined, presumably due to toxicity.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of the test, 4-VCH did not produce any statistically significant increases in sister chromatid exchanges in CHO cells with or without activation at any of the concentrations tested, nor was there a positive dose-related trend.

Reliability: (2) valid with restrictions
Comparable to guideline study. Data tables and briefly reported methods/results available for review, acceptable for evaluation.

10-JUL-2006 (49)

Type: Chromosomal aberration test
System of testing: Chinese Hamster Ovary (CHO) cells in vitro
Concentration: 25 to 149.5 ug/mL and 12.5 to 99.8 ug/ml
Metabolic activation: with and without
Result: negative

Method: other: US-NTP standard protocol
GLP: no data

Method: Chinese Hamster Ovary (CHO) cells were incubated for 8-12 hours with the test chemical in supplemented McCoy's 5A medium; colcemid was added and incubation continued for 2 hours.

The incubation time and the dose levels selected were determined from the information on cell cycling and toxicity obtained from the SCE test; if cell cycle delay was anticipated in the CA test, the incubation period was extended to permit accumulation of sufficient cells in first metaphase for analysis.

In experiments without S9 activation, cells were harvested after 10.5 hours treated or after 12.5 hours for incubations

in the presence of S9 activation. Cells were then harvested fixed, and stained with Giemsa.

Mitomycin C used as a positive control for tests performed without S9 activation, cyclophosphamide for tests performed with S9 activation.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 +/- 2 chromosomes). One hundred (100) first-division metaphase cells were scored at each dose level. Aberrations were recorded as "simple" (breaks and terminal deletions), "complex" (rearrangements and translocations), and "other" (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Statistical analyses were conducted to assess the presence of a dose-response (trend test) and the significance of the individual dose points relative to the vehicle control. For a single trial, a statistically significant ($P<0.05$) difference for one dose point and a significant trend ($P<0.015$) was considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive.

Result: A 0% to 4% increase in total abberations was recorded across the various test concentrations, with and without S9 activation, compared to 0% to 1% in the negative and vehicle controls. The response was not dose-dependent.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of the test, 4-VCH did not produce any statistically significant increases in total abberations, complex abberations, simple abberations, or other abberations in CHO cells with or without activation at any of the concentrations tested.

Reliability: (2) valid with restrictions
Comparable to guideline study. Data tables and briefly reported methods/results available for review, acceptable for evaluation.

10-JUL-2006 (48)

Type: other: various in vitro tests

Remark: Information presented below refers to 4-VCH metabolites:

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 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.

Test substance: Other test substance: metabolites of 4-VCH.

Reliability: (4) not assignable

31-MAY-2006 (30)

5.6 Genetic Toxicity 'in Vivo'

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of admin.: inhalation
Exposure period: 13 wk
Doses: 0, 50, 250, and 1000 ppm
Result: negative

Method: EPA OTS 798.5395
GLP: yes

Method: Groups of 5 male and 5 female B6C3F1/CrlBR mice (Charles River Breading Laboratories), approximately 5 weeks old on the first day of treatment, were exposed whole body to 0 (air), 250, 1000 or 1500 ppm 4-VCH for 6 hours/day, 5 days per week for 13 weeks. A positive control group of 5 male and 5 female mice were exposed concurrently to 1000 ppm of 1,3-butadiene.

They were fed Purina® Certified Rodent Chow (chunk) #5002 and tap water ad libitum when in their home cages.

Chamber concentrations were verified by GC-FID at approximately 30-minute intervals. Temperature and relative humidity within the chambers were similar to housing conditions (target: 22 degrees C, 40% RH).

Animals were observed regularly for clinical signs, morbidity or abnormal behavior and appearance. Body weights were recorded prior to the first exposure, at weekly intervals thereafter, and prior to sacrifice.

Approximately 24 hours after the final exposure, animals were sacrificed (carbon dioxide), the femurs removed and bone marrow smears prepared (Miniprep® automatic blood smearing instrument). At least two slides per animal were prepared, fixed in methanol and stained with acridine orange in phosphate buffer (pH 7.4). Good quality cell preparations were examined (blind) using incident light fluorescence microscopy, and the proportion of PCEs among 1000 erythrocytes (PCE frequency) and the proportion of MN PCEs among 1000 PCEs (MN PCE frequency) were determined.

Data for PCE frequency and MN PCE Frequency were transformed

prior to statistical analysis using arcsin square root transformation. If the transformed data was normally distributed, parametric methods were used for statistical analysis; if not, nonparametric methods (Kruskal-Wallis test, Mann-Whitney U test) were applied to the non-transformed data. Body weight gain data were analyzed using parametric methods.

Result:

Purity of test samples
Laboratory analysis of the test substance and positive indicator compound (1,3-butadiene) at the start of the study and again at the end indicated that the composition of the test materials were unchanged over the course of the study. The purity of the 4-VCH was 99.4% to 99.75% while the purity of 1,3-butadiene was found to be 99.9%. The inhibitor present in both substances was 4-tertbutylcatechol.

Exposure concentrations and chamber conditions
Mean chamber concentrations for 4-VCH over the length of the study (with SD in parenthesis) were 53 ppm (9.7 ppm), 250 ppm (27 ppm), and 1000 ppm (80 ppm) and, for 1,3-butadiene, 980 ppm (140 ppm). Chamber temperatures, humidity, and airflow were reported to be within targeted parameters throughout the study.

Clinical signs of toxicity
All male mice and one-half of the female mice exposed to 1000 ppm 4-VCH were found dead by day 12 of the study. By the end of the study, only 2 female mice survived at this concentration. Tremors and lethargy were observed in 1 male and 2 female mice at 50 ppm, but no clinical signs of toxicity were observed at 250 ppm. All negative control, positive indicator, 50 ppm-exposed, and 250 ppm-exposed animals survived until sacrificed.

Body weight gain
Mean body weight gain data were reported at weekly intervals and over the duration of the study. A statistically significant (alpha = 0.05) decrease in weight gain was reported at 250 ppm for female mice but not at other concentrations for either sex.

Cytogenetic evaluation
The arcsin square root transformed PCE frequency data were found to be normally distributed and, therefore, were analyzed using parametric methods (ANOVA). The mean PCE frequency data were reported as follows:

Conc. (ppm)	Sex	N	Mean(%)	95% Conf. Limits
0	M	5	55.1	49.8, 60.3
50	M	4*	57.5	47.8, 66.9
250	M	5	54.9	49.6, 60.1
1000	M	0	No Data	No Data
1,3-BD	M	4*	61.1	48.1, 73.4

0	F	5	59.8	48.5, 70.6
50	F	5	59.4	50.4, 68.1
250	F	5	60.2	53.2, 67.1
1000	F	2	63.2	47.5, 77.5
1,3-BD	F	5	72.3	64.8, 79.2

The arcsin square root transformed MN PCE frequency data were also found to be normally distributed and, therefore, were analyzed using parametric methods (ANOVA). The mean MN PCE frequency data were reported as follows:

Conc. (ppm)	Sex	N	Mean(%)	95% Conf. Limits
0	M	5	0.14	0.00, 0.50
50	M	4*	0.24	0.12, 0.41
250	M	5	0.33	0.12, 0.66
1000	M	0	No Data	No Data
1,3-BD	M	4*	1.56	0.24, 3.98
0	F	5	0.10	0.00, 0.35
50	F	5	0.14	0.01, 0.43
250	F	5	0.23	0.11, 0.39
1000	F	2	0.19	0.00, 3.58
1,3-BD	F	5	0.78	1.29, 2.10

*Animals were removed from micronucleus study due to technical error.

Given the data presented above, there was no statistically significant depression in the proportion of PCEs among 1000 erythrocytes or increases in MN PCEs in any VCH-treated group, while the positive control group (1,3-butadiene exposed) exhibited a statistically significant elevation of MN PCEs (proportion of PCEs per 1000 erythrocytes unaffected).

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of this study, it can be concluded that 4-VCH did not cause any apparent physiologic or toxic effects on the bone marrow or induce chromosomal or spindle damage in the nucleated erythroblast cells.

Reliability: (1) valid without restriction
Study available for review. GLP-compliant guideline study. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (20)

Type: Micronucleus assay
Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 13 wk
Doses: 0, 250, 1000, and 1500 ppm
Result: negative

Method: EPA OTS 798.5395
GLP: yes

Method: Groups of 5 male and 5 female Crl:CD®BR rats (Charles River Breading Laboratories), approximately 5 weeks old on the first day of exposure, were exposed to 0 (air), 250, 1000 or 1500 ppm 4-VCH 6 hours/day, 5 days per week for 13 weeks. A positive control group of 5 male and 5 female rats received a single intraperitoneal injection of cyclophosphamide USP in sterile water (40 mg/kg body weight) at the end of the 13 week period.

[Other methodological details as for the rat 13 week inhalation exposure micronucleus study described avove.]

Result: Purity of test substance
Laboratory analysis of the test substance at the start of the study and again at the end indicated that the composition was unchanged over the course of the study. The purity was 99.4% to 99.75% with 4-tertbutylcatechol as an inhibitor.

Exposure concentrations and chamber conditions
Mean chamber concentrations for 4-VCH over the length of the study (SD in parenthesis) were reported as 250 ppm (27 ppm), 1000 ppm (80 ppm) and 1500 ppm (79 ppm). Chamber temperatures, humidity, and airflow were reported to be within targeted parameters throughout the study.

Clinical signs of toxicity
Lethargy, clear discharge from the mouth, and stained fur were the most prevalent clinical signs observed and were evident at all 4-VCH chamber concentrations. All animals survived until sacrificed.

Body weight gain
Mean body weight gain was decreased in a dose-related manner in males, which was statistically significant (alpha=0.05) at 1000 ppm and 1500 ppm. For females, body weight gain was decreased but this was not significant (alpha = 0.05) at any exposure concentration.

CYTOGENETIC EVALUATION
The arcsin square root transformed PCE frequency data were found to be normally distributed and, therefore, were analyzed using parametric methods (ANOVA). The mean PCE frequency data were reported as follows:

Conc. (ppm)	Sex	N	Mean(%)	95% Conf. Limits
0	M	5	43.5	29.7, 57.9
250	M	5	49.1	42.7, 55.6
1000	M	5	47.2	35.4, 59.3
1500	M	5	51.6	45.7, 57.5
40mg/kg CP	M	5	25.2	8.9, 46.3
0	F	5	41.3	24.3, 59.4
250	F	5	42.7	35.3, 50.4
1000	F	5	49.7	38.6, 60.8
1500	F	5	48.1	41.2, 55.0
40mg/kg CP	F	5	25.6	18.1, 33.9

The arcsin square root transformed MN PCE frequency data were found not to fit a normal distribution and, therefore, were analyzed using non-parametric methods (Kruskal-Wallis). The mean MN PCE frequency data were reported as follows:

Conc. (ppm)	Sex	N	Mean(%)	Std. Error
0	M	5	0.08	0.05
250	M	5	0.08	0.04
1000	M	5	0.16	0.09
1500	M	5	0.06	0.04
40mg/kg CP	M	5	0.96	0.20
0	F	5	0.20	0.10
250	F	5	0.16	0.06
1000	F	5	0.12	0.06
1500	F	5	0.10	0.08
40mg/kg CP	F	5	0.78	0.12

Given the data presented above, there was no statistically significant depression in the proportion of PCEs among 1000 erythrocytes or increases in MN PCEs in any VCH-treated group, while the positive control group (CP-treated) exhibited a statistically significantly elevation of MN PCEs. The proportion of PCEs among 1000 erythrocytes was also depressed among the positive controls but this was not noted as statistically significant.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of this study, it can be concluded that 4-VCH did not cause any apparent physiologic or toxic effects on the bone marrow or induce chromosomal or spindle damage in the nucleated erythroblast cells.

Reliability: (1) valid without restriction
Study available for review. GLP-compliant guideline study. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (20)

5.7 Carcinogenicity

Species: rat **Sex:** male/female
Strain: Fischer 344
Route of administration: gavage
Exposure period: 103 wk
Frequency of treatment: consecutive days
Post exposure period: none
Doses: 0, 200 or 400 mg/kg bw/d
Result: ambiguous
Control Group: yes, concurrent vehicle

Method: other: standard NTP methodology
GLP: no data

Method: Groups of 50 male and 50 female F344 rats (Charles River Breeding Laboratories ; age 7 wk at start of treatment) were administered 4-VCH (>98% pure) in corn oil by gavage at doses of 0, 200 or 400 mg/kg bw/d 5 d/wk for 103 wk. Dose volume = 3.33 ml/kg.

[Other methodological details as reported above for the rat 103 wk chronic study (see section 5.4).]

Statistical methods:
- survival analyses
The probability of survival was estimated using the procedure of Kaplan and Meier, with dose-related effects analyzed by the methods of Cox and of Tarone. (Animals dying from non-natural causes or missing from the study were excluded.) Where differences were present, additional analysis was carried out to identify the time point at which differences became significant.

- analysis of tumor incidence
Results were analyzed using life table analysis (method of Cox and of Tarone), incidental tumor analysis (computational methodology of Haseman) and unadjusted incidence analysis (based on Fisher exact test and Cochran-Armitage linear trend test).

- historical control data
Historic control tumor incidences from the NTP database were used in some instances to assist interpretation.

Result: GC-FID analysis demonstrated that approx. 94% of the dosing solutions were within specification during the study.
[See rat 103 wk study, section 5.4, for further details.]

Body weight and clinical signs
Body weight was decreased 5-14% in high dose males from study wk 72.
[See rat 103 wk study, section 5.4, for further details.]

No clinical signs were described.

Survival
Survival of high dose males was significantly lower than that of controls from wk 5, and for low dose males from wk 88. Overall survival of high dose females was also lower than controls.
[See rat 103 wk study, section 5.4, for further details.]

Tumor pathology
Neoplastic lesions present in skin, urinary bladder, pituitary, preputial gland and clitoral gland.

- skin
Squamous cell papillomas and squamous papillomas or carcinomas (combined) occurred with a significant positive trend in male rats. First recorded between study wk 60-88 with an average of 23 wk between detection and death. Incidence by dose level:

Overall rate:	0%,	2%,	6%
Adjusted rate:	0%,	3.6%,	31.9%
Terminal rate:	0%,	0%,	20%

- urinary bladder
A transitional cell papilloma was present in 1/47 high dose females, and a transitional cell carcinoma in 1/49 low dose females (males unaffected). Comment: the report notes these are rare tumors, with a historical incidence of 3/1084 (0.3%; corn oil vehicle females).

- anterior pituitary gland
Incidence of adenoma or adenoma and carcinoma (combined) increased significantly (life table test) in low dose females only. Incidence for adenoma and carcinoma (combined), by dose level:

Overall rate:	38%,	50%,	16%
Adjusted rate:	44.9%,	66.0%,	39.9%
Terminal rate:	43%,	56%,	23%

- preputial gland
Incidence of adenoma or carcinoma (combined) increased with a positive trend (life table test), although incidence in the high dose groups did not differ from controls. Incidence by dose level:

Overall rate:	2%,	2%,	6%
Adjusted rate:	2.4%,	5.3%,	20.9%
Terminal rate:	0%,	0%,	0%

- clitoral gland
Incidence of adenoma or squamous cell carcinoma (combined) increased significantly (life table test, incidental tumor test) in low dose females only. Incidence by dose level:

Overall rate:	2%,	10%,	0%
Adjusted rate:	2.5%,	17.9%,	0.0%
Terminal rate:	3%,	18%,	0%

Remark

When reviewing results from this study, the NTP Peer Review Panel concluded that interpretation of the study findings was confounded by poor health and low survival of the animals.

It is also noted in the report that the apparent statistical significance of some tumors may have reflected their earlier detection in rats dying of unrelated/undefined causes rather than due to 4-VCH reducing tumor latency and/or increasing tumor frequency. Conversely, it also noted that poor survival might have artefactually decreased the occurrence of some late developing tumors.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of this study, gavage (oral) administration of 4-VCH to rats was associated with the occurrence of neoplastic lesions in skin, urinary bladder, pituitary, preputial gland and clitoral gland. Interpretation of these findings is confounded by poor health and low survival which may have resulted in artefactual temporal and statistical associations between treatment and tumor incidence in animals dying of 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.

Reliability: (2) valid with restrictions
Study available for review. Comparable to guideline study, with restrictions. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (50)

Species: mouse **Sex:** male/female
Strain: B6C3F1
Route of administration: gavage
Exposure period: 103 wk
Frequency of treatment: consecutive days
Post exposure period: none
Doses: 0, 200 or 400 mg/kg bw/d
Result: positive
Control Group: yes, concurrent vehicle

Method: other: standard NTP methodology
GLP: no data

Method: Groups of 50 male and 50 female B6C3F1 mice (Charles River Breeding Laboratories ; age 8 wk at start of treatment) were administered 4-VCH (>98% pure) in corn oil by gavage at doses of 0, 200 or 400 mg/kg bw/d 5 d/wk for 103 wk. Dose volume = 3.33 ml/kg.

[Other methodological details as for the rat bioassay (reported above) and the rat 103 wk chronic study (see section 5.4).]

Result: GC-FID analysis demonstrated that approx. 94% of the dosing solutions were within specification during the study.
[See rat 103 wk study, section 5.4, for further details.]

Body weight and clinical signs
Mean body weight was decreased (5-13%) in high dose males between study wk 8-76 only and in high dose females (~5%) from study wk 20 with a 12% weight reduction apparent at termination.
[See mouse 103 wk study, section 5.4, for further details.]

No clinical signs were described.

Survival
Survival of high males decreased significantly relative to controls from study wk 29, and that of high dose females from wk 32.
[See mouse 103 wk study, section 5.4, for further details.]

Tumor pathology
Neoplastic lesions were detected primarily in ovary, lung, hematopoietic system and adrenal gland.

- ovary
Mixed benign tumors, granulosa cell tumors and granulosa cell tumors or carcinomas (combined) occurred in treated female mice with a positive trend and incidence that was significantly greater than in controls irrespective of the method of analysis (i.e. significance unaffected by poor survival). Incidence by dose level:
* mixed tumor, benign

Overall rate:	0%,	52%,	23%
Adjusted rate:	0.0%,	64.1%,	43.3%
Terminal rate:	0%,	63%,	25%

* granulosa cell tumor

Overall rate:	2%,	19%,	23%
Adjusted rate:	2.6%,	23.7%,	47.3%
Terminal rate:	3%,	24%,	38%

* granulosa cell tumor or carcinoma

Overall rate:	2%,	21%,	28%
Adjusted rate:	2.6%,	25.5%,	54.9%
Terminal rate:	3%,	24%,	44%

- lung
Alveolar/bronchiolar adenomas occurred with significant positive trend in males only; incidence in high dose males significantly greater than control (life table test). Incidence by dose level:

Overall rate:	2%,	8%,	6%
Adjusted rate:	2.7%,	9.7%,	30.9%
Terminal rate:	3%,	8%,	29%

- hematopoietic system
Malignant lymphoma occurred in male mice with significant positive trend; significantly increased in high dose males after adjustment for survival. Incidence by dose level:

Overall rate: 8%, 14%, 10%
Adjusted rate: 10.5%, 16.7%, 62.5%
Terminal rate: 8%, 13%, 57%

-adrenal gland
Capsular adenomas detected with significant positive trend in female mice; significantly increased incidence in the high dose group (life table test). Incidence by dose level:
Overall rate: 0%, 6%, 8%
Adjusted rate: 0.0%, 7.7%,18.3%
Terminal rate: 0%, 8%, 12%
Comment: the report notes that these lesions may be secondary to ovarian tumors described above.

Remark
The report notes that early death of the majority of high dose male mice confounds interpretation of hematopoietic and lung findings. Since tumor incidences were not altered in low dose males, the apparent statistical significance achieved in the high dose group may reflect earlier detection in animals dying of unrelated/undefined causes rather than as a result of reduced tumor latency and/or increased tumor frequency. Conversely it also noted that poor survival might have also artefactually decreased the occurrence of some late developing tumors. Overall, NTP concluded that the study was inadequate and the results inconclusive with regard to the potential carcinogenicity of 4-vinylcyclohexene in male mice.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of this study, gavage (oral) administration of 4-VCH to mice was associated with the occurrence of neoplastic lesions in ovary, lung, hematopoietic system and adrenal gland. Interpretation of findings for males (lung, hematopoietic system) is confounded by poor health and low survival which may have resulted in artefactual temporal and statistical associations between treatment and tumor incidence in animals dying of unrelated/undefined causes. In females, 4-VCH significantly increased the incidence of several types of uncommon ovarian tumors in both dose groups in a manner that was independent of survival. The incidence of adrenal gland tumors was also increased in females, however it was unclear if this was a direct effect of 4-VCH or secondary altered ovarian function. Overall, NTP concluded that the occurrence of ovarian tumors provided clear evidence of potential carcinogenicity of 4-vinylcyclohexene in the mouse.

Reliability: (2) valid with restrictions
Study available for review. Comparable to guideline study, with restrictions. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (15) (50)

Remark: The carcinogenicity of 4-VCH has been considered by IARC.

Administration of 4-VCH by gastric intubation produced granulosa-cell and mixed tumors of the ovary and adrenal subcapsular tumors in female mice. In male mice, there was an increase in the incidence of lymphoma and lung tumors.

Following gastric intubation in rats, increased incidences of squamous-cell tumors of the skin in males and of clitoral gland tumors in females were onserved.

IARC Evaluation:
There is inadequate evidence in humans for the carcinogenicity of 4-vinylcyclohexene.

There is sufficient evidence in experimental animals for the carcinogenicity of 4-vinylcyclohexene

Overall evaluation: 4-vinylcyclohexene is possibly carcinogenic to humans (Group 2B).

31-MAY-2006 (29)

Species: mouse **Sex:** male
Strain: Swiss
Route of administration: dermal
Exposure period: Not specified: lifetime, median survival 54 weeks.
Frequency of treatment: 3 applications per week
Post exposure period: none
Doses: Approx. 54 mg of test substance per painting
Result: ambiguous
Control Group: other: various included, see methods

Method: Thirty (30) male Swiss Mice (Millerton Research Farms), approximately 8 weeks old on the first day of exposure, were painted 3 times per week with 4-VCH in 50% benzene using an artist's watercolor brush, which reportedly delivered 45 mg of solution per application. The entire backs of the animals were painted. When necessary, the hair was clipped. The test solution was reported to be of commercial quality (K&K Laboratories) and purified with aqueous ferrous sulfate followed by distillation in a nitrogen atmosphere to remove oxidation products. Purity was verified prior to the study by vapor phase gas chromatography, with no indication of similar testing at the end of the study.

Four types of control groups were included in the study: 1) groups that received 3 paintings per week of 100 mg of benzene only (100% benzene), 2) groups that received 3 paintings per week of 100 mg of acetone only (100% acetone), 3) positive control groups that received 100 mg of benzo[a]pyrene (BaP) solution at 0.01% in either benzene (0.1% BaP in benzene) or

acetone (0.1% BaP in acetone), and 4) groups receiving no treatment (no treatment).

Tumors were excised at death and confirmed microscopically.

For each compound tested and for the untreated controls, the total tumor and malignant tumor indices were calculated and defined as 10,000 times the reciprocals of the computed time in days to produce tumors in 50 percent of mice using life-table analysis. Thus, a compound that produced tumors, benign or malignant, in 50% of the mice after 100 days and cancers in 50% of the mice after 200 days would have a total tumor index of 100 and a malignant tumor index of 50.

No statistical analysis was reported.

Result: CLINICAL SIGNS OF TOXICITY
Extensive skin damage was reported in the 4-VCH exposed group.

TUMOR EVALUATION
The number of animals tested (n), median survival time (ST), total number of tumors (TT), total number of cancers (TC), total tumor index (TTI), and malignant tumor index (MTI) for the test substance and control groups, were reported as follows:

Substance	n	ST	TT	TC	TTI	MTI
4-VCH in:						
50% Benzene	30	375	6	1	13	10
100% Benzene	30	264	2	0	10	<10
100% Benzene	30	262	5	0	19	<10
100% Benzene	30	412	2	0	<10	<10
100% Benzene	60	292	2	1	<10	<10
100% Acetone	30	240	2	0	10	<10
100% Acetone	30	652	0	0	<10	<10
100% Acetone	30	330	4	0	14	<10
100% Acetone	30	134	2	0	18	<10
0.01% BaP in:						
Acetone	30	211	16	7	50	32
Acetone	30	378	24	20	34	29
Acetone	30	240	25	11	45	27
Acetone	30	259	18	11	45	36
0.01% BaP in:						
Benzene	30	351	16	7	42	27
Benzene	30	348	10	6	24	21
Benzene	30	370	23	13	36	26
No Treatment	30	175	2	1	14	<10
	30	342	0	0	<10	<10
	30	730	0	0	<10	<10

30	624	0	0	<10	<10
30	217	5	0	20	<10
30	112	1	0	<10	<10
28	253	4	0	<10	<10
60	345	1	0	<10	<10

During the year prior to the study, the background incidence of tumors in the Swiss-Millerton male mice was 1.4% and were reported to be all benign squamous papillomas.

During the study, tumors (benign and malignant) were observed in 20% of mice treated with the 4-VCH in 50% benzene solution, in 6.7% of the mice treated with benzene only, and in 5% of untreated mice. The malignant tumors observed were reported to be squamous cell cancers and occurred in the test population as well as controls.

During part of the study, ectromelia was present in experimental and control groups and, as a result, animals had to be vaccinated against the disease.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of this study, it can be concluded that 4-VCH in a 50% solution of benzene resulted in an increased number of benign squamous cell papillomas when painted on the skin of male Swiss mice. The one malignant tumor observed in the group treated with 4-VCH was not thought to be necessarily attributable to the test substance, but instead was attributed to speculative formation of 4-VCH hydroperoxide in the test substance via autoxidation in air.

Reliability: (2) valid with restrictions
Study available for review. Pre-guideline, pre-GLP investigation. Acceptable for assessment.

10-JUL-2006 (66)

5.8.1 Toxicity to Fertility

Type: other: continuous breeding study
Species: mouse
Sex: male/female
Strain: CD-1
Route of administration: gavage
Exposure Period: continuous
Frequency of treatment: once daily
Doses: 0, 100, 250, and 500 mg/kg/day (F0); 0 and 500 mg/kg/day (F1)
Control Group: yes, concurrent vehicle

Method: other: US-NTP Continuous Breeding Protocol
GLP: yes

Method: Male and Female CD-1 (ICR)BR outbred Swiss albino mice (VAF/plus; Charles River Breeding Laboratories, Inc., Raleigh, NC), approximately 9 weeks old upon arrival, were used for

this study. They were fed deionized and filtered water and pelleted food (NIH-07, Zeigler Brothers, Gardners PA) ad libitum, and housed in environmentally controlled conditions (72 degree F, 53% RH, 14 hr light/10 hr dark cycle).

At age 11 weeks, animals for the F0 generation were assigned to treatment groups and administered 0, 100, 250 or 500 mg/kg body weight/d 4-VCH in corn oil by gavage. There were 40 male and 40 female control mice, with 25 per sex in each 4-VCH treatment group. During the first week of treatment, animals were housed in pairs by sex by dose group, then in breeding pairs within dose groups during weeks 2-15. Pups born during this time were euthanized immediately after examination. At week 16, the F0 breeding pairs were separated and the dams allowed to deliver and rear their final litter (F1 generation) to PND 21. Food and water consumption and body weight data were collected during weeks 1, 2, 5, 9, 13 and 18 (females).

For the F1 fertility assessment, 21 day old pups (20 males, 20 females) from the control and the high-dose groups were housed in same sex pairs and treatment with 4-VCH begun the following day. At approximately 74 days of age, the animals were allocated to nonsibling breeding pairs for up to 7 days and the females allowed to litter. Feed and water consumption were measured during weeks 1 (breeding), 2, 3, and 4.

Parent (F0) cohabitation parameters included: date of delivery of each litter, number, sex, weight of pups per litter, number of litters per pair, and PND 0 dam body weight. On PND 0, 4, 7, 14, and 21, surviving pups were counted, sexed, and weighed for all dams delivering a litter after week 16.

F1 generation cohabitation parameters included: date of delivery of each litter, number, sex, weight of pups per litter, number of litters per pair, and PND 0 dam body weight. After delivery of the litters, vaginal smears were collected daily for 12 days. At study end, F1 parents were subject to necropsy and body wieght, kidney/adrenal weights, liver, testis, prostate, seminal vesicle (+ coagulating gland), ovary/oviduct and uterus weights collected. The ovaries were processed for microscopic assessment. Sperm parameters (including sperm motility, concentration, morphology) and homogenization-resistant spermatid concentration were also recorded.

Data were analysed using Williams'modification of Dunn's or Shirley's nonparametric multiple comparison procedures.

Result: Survival
Five (5) parental generation (F0) animals reportedly died during F0 cohabitation, including 2 out of 40 control males, 1 out of 40 control females, and 2 out of 20 females from the high-dose group from indeterminate causes. Seven (7) animals were removed from the study due to gavage-related injuries and 4 for cage-mate inflicted fatalities. This brings the total

number of animals excluded from the study to 16. However, the total number of breeding pairs reported was 36 control pairs, 19 low-dose pairs, 19 mid-dose pairs, and 16 high-dose pairs for a total of 180 out of 200 animals included in the study. The 4 animals not accounted for are presumed to be the cage mate of an animal that was removed for cause.

Five (5) F1 animals died during the F1 fertility assessment phase, including 1 control male and 3 males and 1 female from the high dose group from indeterminate causes. A total of 18 control and high dose animals were injured during gavage dosing and had to be removed from the study. Most were removed within 1 week after weaning. Despite these loses, presumably because most or all occurred prior to the selection of pairs for cohabitation, a total of 20 control and 19 treated pairs appear to have survived the study.

Parental effects
4-VCH at all treatment doses had no effect on reproductive competence including initial fertility, litters per pair, live pups per litter, total pups born alive, proportion of pups born alive, and sex ratio of pups. High-dose females exhibited slight general toxicity evident as an 8% reduction in body weight compared to controls (data not reported). A 4% decrease in body weight was also reported to be statistically significant but only among the high-dose group where the total number of surviving females was reduced from 20 to 16. Preweaning growth and survival were not affected and, when adjusted for the number of pups per litter, the reduction in pup weights was no longer significant. Other than some transient increases in water consumption in the low and high dose groups during weeks 5, 9 and/or 27, no significant effects were observed regarding food and water intake. Data are as follows:

Parameter	-- Dose (mg/kg bwt/d) --			
	0	100	250	500
No. fertile/No. Cohabitated	36/36	19/19	19/19	16/16
Litters per pair	4.8	4.7	4.8	4.6
Live pups per litter	12.2	13.5	12.5	11.5
Pups born alive (%)	97	99	99	99
Live males per litter	49	48	48	49
Live pup weight (g)	1.64	1.58	1.58	1.58*
Adjusted live pup weight (g)	1.63	1.61	1.58	1.55

*Reported as statistically significant

F1 body weight effects
Body weights of Male and Female F1 pups born after the end of F0 cohabitation (Week 16)on postnatal days (PND) 0, 7, 21, 77, and 117 were slightly reduced when compared to controls but only the reductions observed at weeks 77 and 117 were identified as statistically significant. This may be attributable to the different statistical method employed at

this stage of the study. Male/Female F1 body weights (g) were reported as follows:

	Male/Female (g)			
PND	Dose (mg/kg bwt/d)			
	0	100	250	500
0	1.75/1.66	1.69/1.59	1.75/1.64	1.63/1.59
7	4.38/4.27	4.35/4.08	1.75/4.22	4.23/4.08
21	11.07/10.3	10.98/9.36	10.79/10.20	10.94/10.56
77	34.07/28.4	---/---	---/---	31.51*/26.20*
117	35.24/.0.6	---/---	---/---	32.79*/28.00*

*Reported as statistically significant

Fertility and reproductive performance
4-VCH at all treatment doses had no effect on reproductive competence including mating index, fertility index, gestation length, live F2 pups per litter, total number of F2 pups born alive, total number of F2 male pups per litter, or live F2 pup weight. Data were presented as follows:

	Dose	
	--- mg/kg bwt/day ---	
Parameter	0	500
Mating index^	16/20	18/20
Fertility index^^	19/20	19/20
Number of days to litter	18.7	19.2
Live F2 pups per litter	11.6	10.6
F2 pups born alive	99	98
F2 male pups born alive	41	39
Live F2 pup weight	1.52	1.47

^Number of females vaginal plug positive / number cohabitated
^^Number of females delivering a litter / number cohabitated

F1 relative organ weights
4-VCH caused a statistically significant increase in liver weights in F1 males (55.59 +-1.2 for controls vs. 60.46 +-1.37 for treated) and in F1 females (57.52 +-1.18 for controls vs. 62.08 +-1.28 for treated) at necropsy compared to controls. All other organ weights assessed were considered normal. Twenty (20) male controls, 20 female controls, 19 male high-dose, and 20 female high-dose were evaluated.

F1 sperm analysis
4-VCH had no effect on epididymal sperm concentration or morphology, but did cause a statistically significant increase in sperm motility and a statistically significant decrease (16%) in spermatid concentration in the right testis homogenates. No histopathologic lesions were noted for the testis. Data were reported as follows:

	mg/kg bwt/day	
Parameter	0	500
	(n=20)	(n=19)

Epididymal sperm concentration	988	876
Epididymal sperm motility	68.9	85.5*
Epididymal sperm morphology	2.4	2.9
Testicular sperm concentration	13.6	11.3*

*Reported as statistically significant

F1 vaginal cytology
4-VCH had no effect on normal cyclic patterns of vaginal cytology or mean cycle length following approximately 95 days of exposure to 500 mg/kd bw/day.

F1 sectioned ovary results
4-VCH at 500 mg/kg bw/day for approximately 95 days caused a statistically significant reduction in the number of primordial oocytes/folicles by 33%, the number of growing follicles by 55%, and the number of antral follicles by 33%. Data were reported as follows:

	mg/kg bwt/day	
Follicular stage	0	500
	(n=20)	(n=19)
Primordial oocytes/follicles	208.9	140.6*
Growing follicles	51.2	23.2*
Antral follicles	7.4	4.95*

*Reported as statistically significant

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of this study, 4-VCH administered at 500 mg/kg bw/day was clearly toxic to ovarian follicles in female offspring and produced a slight but statistically significant effect on spermatogenesis in male offspring, but did not adversely affect reproductive performance in either the F0 or F1 generations.

Reliability: (1) valid without restriction
Study available for review. GLP-compliant near-guideline study. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (24)

5.8.3 Toxicity to Reproduction, Other Studies

Type: other: ovarian toxicity
In Vitro/in vivo: In vivo
Species: mouse
Strain: B6C3F1 **Sex:** female
Route of administration: i.p.
Exposure period: 30 days
Frequency of treatment: once daily

Method: Groups of female B6C3F1 mice and Fischer 344 rats (Harlan Spargue-Dawley, Indianapolis, IN; age 28 d; n = 4-10 per treatment) received the following daily treatments by i.p. injection in corn oil (2.5 ml/kg body weight) for 30 days:

4-vinylcyclohexene (4-VCH): 0, 100, 400 or 800 mg/kg body weight/day
(equivalent to 0, 0.9, 3.7 or 7.4 mmol/kg/d)

4-vinylcyclohexene diepoxide (4-VCH DE): 0, 10, 40 or 80 mg/kg body weight/day
(equivalent to 0, 0.07, 0.20 or 0.57 mmol/kg/d)

4-vinylcyclohexene-1,2 epoxide (4-VCH 1,2-EP): 0. 0.34, 1.37 or 2.74 mg/kg body weight/day
(equivalent to 0, 0.9, 3.7 or 7.4 mmol/kg/d)

4-vinylcyclohexene-7,8 epoxide (4-VCH 7,8-EP): 0. 0.34, 1.37 or 2.74 mg/kg body weight/day
(equivalent to 0, 0.9, 3.7 or 7.4 mmol/kg/d)

Animals were sacrificed (carbon dioxide) on day 31 and the ovaries removed, fixed (Bouin's solution) and processed (6 um section, H&E staining) for microscopic examination, with oocytes identified and counted.

In other studies, the time course for 4-VCH-induced ovarian damage was investigated in mice (n = 5/treatment) injected with 0 or 800 mg/kg bw/d 4-VCH for 5, 10, 15 or 30 days (ovaries processed as above).

The effect of chloramphenicol (an inhibitor of cytochrome P-450 mediated epoxidation) on 4-VCH-induced damage to the ovary was investigated in female mice (n = 5-6/group) treated by i.p. injection for 15 consecutive days as follows:
Group 1: saline followed by corn oil;
Group 2: chloramphenicol (200 mg/kg body weight in saline) followed by corn oil;
Group 3: saline followed by 4-VCH (800 mg/kg body weight in corn oil);
Group 4: chloramphenicol followed by 4-VCH.
The were administered 1 hr apart using a dose volume of 2.5 ml/kg body weight/day.

On day 16 the animals were sacrificed and the ovaries processed (as above) for histological assessment.

Dose-response curves were obtained by non-linear regression, and the ED50 (defined as dose reducing the oocyte number to 50% of control) calculated. Significant differences between curves were analyzed using the sum of squares of the two data sets under comparison and as a single pool to calculate an F value. Student's t-test was used to determine the significance of differences between group means while multiple comparisons used one-way ANOVA and the Newman-Kuels range test.

Result: Graphical data demonstrate clear differences in response between rats and mice to the ovarian toxicity associated with 4-VCH. In mice, small oocyte counts were decreased in a dose-dependent manner from around 300/ovary in controls to 50-100/ovary in treated animals given 800 mg/kg bw/d by i.p. injection for 30 days; oocyte numbers in rats, in contrast, were unaffected (approx. 150 oocytes/ovary).

The epoxides and diepoxide of 4-VCH were more potent ovotoxins, and all markedly reduced oocyte numbers in both rats and mice in a dose-related manner.

The ED50 values for oocyte reduction were calculated as follows:

	ED50 (mmol/kg/day)			
	4-VCH	4-VCH 1,2EP	4-VCH 7,8-EP	4-VCH DE
Mouse	2.7	0.5	0.7	0.2
Rat	>7.4 (a)	1.4	ND (b)	0.4

a = highest dose given
b = not done

Time course studies revealed no significant reduction in oocyte numbers in mice given 800 mg/kg/d 4-VCH until after 15 days treatment after which time number continued to decline:

Day	Small oocyte count (approx. % of control)
5	100
10	84
15	35
30	8

(Values obtained by interpolation from graphical data.)

The oocyte loss induced by 4-VCH was partially overcome by pre-treatment of female mice with chloramphenicol:

	Small oocyte count
Controls	100% (a)
Saline / 4-VCH	38% *
Chloramphenicol / 4-VCH	58% *

a = data for saline and chloramphenicol-pretreated control groups combined for statistical analysis
* P<0.05
(Values obtained by interpolation from graphical data.)

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Results from these investigations demonstrate species differences in the ovarian toxicity of 4-VCH, with mice (ED50 = 2.7 mmol/kg body weight/day) more sensitive than rats (ED50 not established; > 7.4 mmol/kg body weight/day, the highest dose tested). Both species, in contrast, were sensitive to the epoxide- and diepoxide metabolites of 4-VCH (ED in range 0.2-1.4 mmol/kg body weight/day). 4-VCH-dependent oocyte loss was reduced in mice pre-treated with chloramphenicol, an inhibitor of epoxide hydrolase.

Reliability: (2) valid with restrictions
Study available for review. Non-guideline experimental study. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (58)

Type: other: ovarian toxicity
In Vitro/in vivo: In vivo
Species: mouse
Strain: B6C3F1 **Sex:** female
Route of administration: i.p.
Exposure period: 30 days
Frequency of treatment: once daily

Method: Female B6C3F1 mice (Harlan, Inc., Indianapolis, IN; approximately 21 days old on delivery) were housed five per cage in sawdust bedding and given food (Teklad, Harlan Sprague_Dawley, Inc. Madison, WI) and water ad libitum. The animal room was maintained on a 12 hr light/dark cycle and animals were allowed to acclimatize for 7 days before use.

At age approximately 28 days, groups of mice (n=15/group) were administered sesame seed oil (vehicle control), 4-VCH (650 mg/kg 4-VCH in sesame seed oil) or 4-phenylcyclohexene (4PC; 475 or 950 mg/kg in sesame seed oil) once daily by i.p. injection for 30 days. As a positive control, a group of 10 mice was treated with 80 mg/kg benzo[a]pyrene (BaP) on the first day of dosing and again 7 days later.

On a daily basis, animals were weighed and vaginal smears were collected to determine the stage of estrus. On the first day of diestrus, the animals were euthanized via CO2 asphyxiation. Blood was collected from the posterior vena cava and plasma was separated and frozen for determination of follicle-stimulating hormone (FSH) concentrations. Ovaries were removed and fixed in Bouin's solution for 24 hours followed by immersion in 70% ethanol. Ovaries were then processed, embedded in paraffin, step-sectioned at 5 to 6 um, and stained with hematoxylin and eosin. Every 20th section of the right ovary of each mouse was examined to determine the

number of small and growing follicles according to the method of Pederson and Peters (1968).

Statistical analysis was performed using the Number Cruncher Statistical System 5.0 (NCSS Kaysville, UT). Differences were considered significant when $p < 0.05$.

Result: Daily dosing with 4-VCH was resulted in reductions in the numbers of small and growing follicles, as did the two doses of the positive control compound, but not the vehicle control or treatment with 4PC. The authors report that, in most sections, the follicles were completely absent. Although no specific data were presented, the number of small follicles per ovary (SFO) and the number of growing follicles per ovary (GFO) are estimated, using a ruler and the bar chart presented, as follows:

Group	SFO	GFO
Control	275	115
4PC(low-dose)	274	115
4PC (high-dose)	252	113
4-VCH	30*	32*
BaP	42*	50*

* P<0.05

There were no statistically significant reductions reported in the concentrations of plasma follicle-stimulating hormone (FSH) observed in treatment groups when compared to controls:

Control	100%
4PC (low-dose)	92%
4PC (high-dose)	108%
4-VCH	85%
BaP	192%

Values obtained by interpolation from graphical results presented in the paper.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Under the conditions of this study, 4-VCH administered at 650 mg/kg bw/day by i.p. injection for 30 days was clearly toxic to ovarian follicles but did not result in a statistically significant reduction in plasma follicle-stimulating hormone.

Reliability: (2) valid with restrictions
Study available for review. Non-guideline research investigation containing limited data, acceptable for evaluation.

10-JUL-2006 (27)

Type: other: ovarian toxicity
In Vitro/in vivo: In vivo
Species: mouse
Strain: B6C3F1 **Sex:** female

Method: Groups of female B6C3F1 mice (age 28 days) were administered 4-VCH (7.5 mmol/kg body weight; positive control), sesame seed oil (2.5 ml/kg body weight; vehicle control) or a series of structural analogues by i.p. injection for 30 days:

	mmol/kg body weight/day
4-VCH	7.5
Ethylcyclohexene	7.5
Vinylcyclohexane	7.5
Cyclohexene	7.5
Ethylcyclohexene oxide	1.43
Vinylcyclohexane oxide	1.43
Cyclohexene oxide	1.43
Epoxybutane	1.43
Butadiene monoepoxide	1.43
Butadiene diepoxide	0.14
Isoprene	7.34

Comment: dose selection was either equimolar to 4-VCH, or the maximum tolerated by mice over 30 days (based on preliminary experiments).

Following day 30, mice were sacrificed (carbon dioxide) on the first day of their diestrus cycle (determined from vaginal cytology), the ovaries removed and processed, and every 20th section examined microscopically for enumeration of small- and growing pre-antral follicles present in oocytes.

The ability of the various structural analogues to alkylate nicotinamide in vitro (considered an indicator of the chemical and biological reactivity) was assessed fluorometrically, following published methods (Nelis-Hans et al (1982) Anal Chem 54, 213-216).

Results were analyzed using Student's t- and Newman-Keuls tests.

Result: In an initial series of structure activity studies, none of the analogues of 4-VCH tested lead to a statistically significant decrease in small follicle counts when administered at 7.5 mmol/kg body weight for 30 days, however ethylcyclohexene treatment lead to a clear and significant (-37%) reduction in the number of growing follicles. As expected, 4-VCH markedly and significantly decreased both parameters in female mice.

	-- Follicle counts --	
	Small	Growing
Control (sesame oil)	90	57
Ethylcyclohexene	61	36*

Vinylcyclohexane	98	53
Cyclohexene	66	43
4-VCH	12*	16*

Comment: these compounds contain a single unsaturated site corresponding to either the 1,2 position of 4-VCH (ethylcyclohexene, cyclohexene) or the 7,8 position (vinylcyclohexane): they cannot be metabolized further to a diepoxide. An absence of activity in these experiments suggests that 1,2 or 7,8 mono epoxides are not ovarian toxicants.

Sub-acute treatment of mice with the monoepoxide derivatives of the three analogues (1.43 mmol/kg body weight for 30 days) was without effect on the number of small- and growing follicles in mouse ovary, however a marked reduction in both parameters was again recorded after treatment with 4-VCH (7.5 mmol/kg body weight):

	-- Follicle counts --	
	Small	Growing
Control (sesame oil)	148	43
Ethylcyclohexene oxide	126	30
Vinylcyclohexane oxide	119	33
Cyclohexene oxide	147	50
4-VCH	17*	7*

Comment: these results confirm that monoepoxides corresponding to the 1,2- (ethylcyclohexene oxide, cyclohexene oxide) or 7,8 (vinylcyclohexane) epoxide of 4-VCH were not ovarian toxicants.

In a third series of experiments, isoprene (1.43 mmol/kg), butadiene monoepoxide (1.43 mmol/kg) and butadiene diepoxide (0.14 mmol/kg) (but not epoxybutane, 1.43 mmol/kg) were clearly ovotoxic after repeated administration to female mice, leading to decreases in the number of small and growing follicles comparable to those produced by 4-VCH:

	-- Follicle counts --	
	Small	Growing
Control (sesame oil)	131	51
Epoxybutane	150	42
Butadiene monoepoxide	3*	7*
Butadiene diepoxide	20*	19*
Isoprene	31*	28*
4-VCH	17*	14*

Comment: these findings suggest that biotransformation of olefinic structures to products that are, or that can form, diepoxides is an important requirement for induction of ovarian toxicity. Epoxybutane, despite being a monoepoxide, cannot be metabolized to a diepoxide and was therefore inactive.

The ovarian toxicity of this series of structural analogues was found to correlate with their ability to alkylate nicotinamide in vitro (used as a surrogate indicator of

chemical reactivity in vivo). Graphical results demonstrate that 4-VCH diepoxide (2 mM; activity reported as approx. 150 fluorescence units/hr) was around 3-fold more potent than equimolar levels of cyclohexene oxide, ethylcyclohexene oxide, vinylcyclohexane oxide and 4-VCH 1,2 epoxide in this assay. Alkylation of nicotinamide by butadiene diepoxide (2 mM; activity reported as around 550 fluorescence units/hr) was 3.5 to 10-fold greater than that associated with equimolar levels of butadiene monoepoxide, epoxybutane and isoprene oxide (2-methyl-2-vinyloxirane). These findings suggest a relationship between the chemical reactivity of epoxide and diepoxides in vitro and ovarian toxicity reported in vivo in the mouse.

Test substance: 4-Vinylcyclohexene, CAS No. 100-40-3.

Conclusion: Results from studies using structural analogues of 4-VCH demonstrate that metabolism to the diepoxide is central to induction of ovarian toxicity in the mouse.

Reliability: (2) valid with restrictions
Study available for review. Non-guideline experimental study. Well reported methods and results, acceptable for evaluation.

10-JUL-2006 (18)

5.10 Exposure Experience

Type of experience: other: Measurements of airborne concentrations (area samples)

Remark: Measurements of airborne concentrations of 4-vinylcyclohexene (4-VCH) and other pollutants were obtained in a press room where bias-ply passenger and truck tires were being cured. Sampling was performed using personal air sampling pumps affixed to ladders and equipment to draw workplace air (area samples) at a nominal flow rate of 1.0 to 1.5 liters per minute through glass tubes containing 100 mg of activated coconut shell charcoal in the front section and 50 mg in the back. A total of 9 consecutive 30- to 45-minute samples were collected at 2 locations to represent a 6-hour period during a single shift. The two sampling locations were reported to include the center of the passenger tire curing area and at its periphery away from truck tire curing. Samples were prepared for analysis by placing the charcoal from the front and back sections of the sorbent tube in separate vials then adding approximately 1 micro-liter of carbon disulfide to remove (desorb) any contaminants collected on the charcoal. After gentle swirling and holding for 4 hours, a known quantity of the aliquot (3 to 5 micro-liters) was removed from each vial and injected into a gas chromatograph (GC) equipped with a flame ionization detector (FID) and a suitable separation column. Separate analysis of each backup section suggested that sorbent breakthrough did not occur. Desorption efficiencies and GC performance were also evaluated in the study and found to

be acceptable.

Results:
Arithmetic mean concentrations of 4-VCH were 71.0 ppb in the center of the passenger tire curing area and 92.3 ppb at it's periphery away from truck tire curing.

Conclusions:
From this study, it can be concluded that, historically speaking, exposures to 4-VCH have occurred in the workplace during the curing of bias-ply tires but the nature and extent of these exposures was not comprehensively characterized by this study.

Limitations:
The area measurements obtained in this study may substantially over-estimate or under-estimate actual breathing zone concentrations. In addition, the measurements were made 30 or more years ago and are not expected to be representative or relevant to workplace conditions that would be encountered today. As such, this data is not suitable for rigorous risk assessment purposes.

Reliability: (3) invalid
Study available for review. Significant methodological deficiences.

23-MAR-2006 (53)

Type of experience: other: Measurements of airborne concentrations (area samples)

Remark: Volatile pollutants, including 4-vinylcyclohexene (4-VCH), were sampled and analyzed from workplace air (area samples) associated with several rubber goods manufacturing processes in Italy, including the vulcanization area of a shoe factory, the vulcanization and extrusion areas of a tire re-treading factory, and the extrusion area of an electrical cable insulation plant. Measurements were obtained by using personal air sampling pumps to draw workplace air (area samples) at a nominal flow rate of 1 liter per minute through each of 4 glass tubes containing 500 mg of charcoal arranged in parallel. A total of 35 samples (140 sampling tubes) were collected at the four locations. To minimize the risk of breakthrough, sample durations were limited to 30 minutes. Samples were prepared for analysis by placing the charcoal from the 4 sorbent tubes that constituted each of the 35 samples in seperate screw cap test tubes and then adding approximately 8 mL of trichlorofluoromethane (Freon 11) to remove (desorb) any contaminants collected on the charcoal. After occasional shaking for 1 hour, an internal standard of ethylene glycol ethyl ether acetate in Freon 11 was added. The volume of the solution was then reduced to approximately 0.2 mL by evaporation under a stream of dry helium. Then, a known quantity of the aliquot (approx. 5

micro-liters) was removed from each test tube and injected into a gas chromatograph (GC) - mass spectrometer (MS) equipped with a fused-silica capillary column. Desorption efficiencies and GC performance were evaluated in the study and found to be acceptable.

Results:
The concentration range of 4-VCH measured in each of the 4 sampling locations were reported as follows: 30 to 210 mg/m3 (6.8 ppb to 47.5 ppb) in the shoe sole vulcanization area; non-detected (ND) in the tire re-treading vulcanization area; ND to 3 mg/m3 (ND to 0.68 ppb) in the tire re-treading extrusion area; and ND to 10 mg/m3 (ND to 2.3 ppb) in the electrical cable insulation plant.

Conclusions:
From this study, it can be concluded that, historically speaking, exposures to 4-VCH have occurred in workplaces where rubber goods are vulcanized or extruded but the nature and extent of these exposures was not comprehensively characterized by this study.

Limitations:
The area measurements obtained in this study may substantially over-estimate or under-estimate actual breathing zone concentrations. In addition, the measurements were made more than 20 years ago and may not be representative or relevant to workplace conditions that would be encountered today. As such, this data is not suitable for rigorous risk assessment purposes.

Reliability: (3) invalid
Study available for review. Significant methodological deficiences.

23-MAR-2006 (13)

Type of experience: other: Worker breathing zone measurements

Remark: The airborne concentrations of 4-vinylcyclohexene (4-VCH) were measured in the breathing zones of workers engaged in the production of 1,3-butadiene (BD) and other unspecified downstream products and were reported to the U.S EPA as part of testing consent order negotiations. Actual methods and data are not presented in the report, only a summary of the data.

Results:
One company collected 12 short term (< 30 minute) samples. The average concentration was 0.354 ppm with a range of non-detectable to 2.22 ppm. Thirty-two long term samples were also collected (TWA > 450 min.). The average concentration was 0.03 ppm, with a range of non-detectable to 0.18 ppm. A second company conducted personnel sampling for a seven year period from 1983-1989. Twenty 8-hour TWA samples were collected with an average concentration of

<0.9 ppm. A third company (the BD producer) conducted personnel sampling for a three day period in 1978. The average concentration for the seven samples was 0.04 ppm, ranging from 0.01 to 1.2 ppm.

Conclusions:
From this report, it can be concluded that, historically speaking, exposures to 4-VCH have occurred during the production of BD and downstream products but the nature and extent of these exposures was not comprehensively characterized in this report.

Limitations:
The report does not provide sufficient detail to evaluate the reliability of the data or its relevance to exposures that might be encountered in the workplace today. As such, this data is not suitable for rigorous risk assessment purposes.

Reliability: (4) not assignable
Secondary literature.

27-MAR-2006 (9)

Type of experience: other: Worker breathing zone measurements

Remark: The airborne concentrations of 4-vinylcyclohexene (4-VCH) were measured in the breathing zones of workers engaged in:
- the production of 1,3-butadiene (BD);
- the on-purpose isolation of 4-VCH in the production of vinylnorbornene (VNB) for isomerization to ethylidene norborene (ENB);
- the on-purpose isolation of 4-VCH during the trimerization of BD to produce dodecanedioic acid (DDDA);
- conversion of 4-VCH to mono- and di-epoxide; and
- the inadvertent production of 4-VCH as a byproduct of BD use in rubber production and tire manufacturing. Actual methods and data are not presented in the report, only a summary of the data, which were compiled from questionnaires completed by private companies.

Comment: The data presented in the report is expected to include some of the same data summarized and referenced separately in these robust summaries under: Chemical Manufacturers Association (CMA) (1990) Report on the survey of the Butadiene Panel of the Chemical Manufacturers Association on 4-Vinylcyclohexene. Submitted to the U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, D.C. May 3, 1990.

Results:
The concentration of 4-VCH measured in the worker's breathing zone and representing full-shift time-weighted average exposures, were reported as follows:
- BD Production: 4 companies reporting; 110 samples; Range <0.01 to <0.04 ppm

- On-Purpose Isolation: 2 companies reporting; 95 samples; Range <0.01 to 1.2 ppm
- Conversion to Epoxide: 1 company reporting; 19 samples; Range <0.01 to 0.09 ppm
- Rubber Production: 10 companies reporting; 411 samples; Range <0.01 to 1.2 ppm
- Tire Manufacturing: 3 companies reporting; 24 Samples; Range 0.002 to 0.02 ppm

Conclusions:
From this report, it can be concluded that historical exposures to 4-VCH have generally been below 1 ppm, as an 8-hour time-weighted average in the industry sectors surveyed, but the nature and extent of exposures occurring at each facility was not comprehensively characterized.

Limitations:
The report does not provide sufficient detail to evaluate the reliability of the data or its relevance to exposures that might be encountered in the workplace today. As such, this data is not suitable for rigorous risk assessment purposes.

Reliability: (4) not assignable
Secondary literature.

23-MAR-2006 (10)

Type of experience: other: Measurement of carpet emisisons

Remark: The emissions of volatile organic compounds, including 4-Vinylcyclohexene (4-VCH), were quantified from new carpets placed in a large-scale (20 cubic meter) environmental chamber. Four different carpets were studied, including 2 that incorporated a styrene-butadiene (SB) latex backing adhesive. No pads or adhesives were used. The carpets selected were reported to be representative of the types used in residences, schools, and offices. Carpets were obtained directly from the finishing line at the manufacturer's mills, sealed in Tedlar bags, and shipped by air freight for delivery to the laboratory. The large chamber was insulated and environmentally controlled, with all interior surfaces clad in stainless steel. Air presented to the chamber was filtered and tested to ensure no outside contaminants were inadvertently introduced. The chamber was operated to ensure 1 air-change per hour with air velocities of 6.5 to 9 cm/sec. at a temperature of 22.8 to 23.5 °C and a relative humidity of 46.5 to 50.2%. Air samples inside the chambers were obtained at approximately 1, 3, 6, and 12 hours after closing the chamber, then again at 24 hours, using multi-sorbent samplers packed with Tenax-TA, Ambersorb XE-240, and activated charcoal in series. Air flow rates through the sorbent tubes was 50 to 200 cubic centimeters per minute, with sample volumes of 1.25 to 10 liters. Samples were then thermally desorbed,

concentrated, and introduced into a capillary gas chromatograph with a mass spectrometer detector (GC/MS). In the field study, samples were collected and quantified for only 2 analytes, which did NOT include 4-VCH.

Results:
The two carpets with the SB latex adhesive emitted, in order of decreasing emission rates, styrene, 4-phenylcyclohexene, 4-VCH, and alkyl benzenes followed by other organic compounds. The concentration of 4-VCH in the chamber ranged from approximately 6 ppb to 17 ppb during the first hour, 3 ppb to 14 ppb during the 3 hour, and 2 ppb to 7 ppb during the 6th hour. The emission rates calculated for 4-VCH ranged from 7.3 to 24.2 micrograms per square meter per hour during the first 24 hours, and 0.6 to 2.7 micrograms per square meter per hour over the entire 7 day test period. The concentration of 4-VCH decayed by 89 to 91% from the first 24 hours to the end of the experiment 7 days later.

Conclusions:
From this study, it can be concluded that historically carpets that incorporate a styrene-butadiene backing adhesive have emitted 4-VCH at levels lower than other contaminants such as styrene and 4-phenylcyclohexene.

Limitations:
The results of this study are historical in nature and do not fully describe the nature and extent of exposures to 4-VCH from carpets manufactured today and installed in typical occupied spaces. This data is suitable for screening level risk assessments, but may not be suitable for a rigorous risk assessment.

Reliability: (1) valid without restriction
Study available for review. Test procedure in accordance with generally accepted scientific standards. Adequately reported methods and results, acceptable for evaluation.

27-MAR-2006 (26)

(1) ACGIH (2001) 4-VINYL CYCLOHEXENE, Cas number 100-40-3. Documentation for TLV.

(2) American Conference of Governmental Industrial Hygienists TLVs and BEIs (2005) Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. Cincinatti, OH, p59.

(3) American Industrial Hygiene Association (2006) The AIHA 2006 Emergency Response Planning Guidelines and Workplace Environmental Exposure Level Guides Handbook. American Industrial Hygiene Association. Fairfax, VA, p39.

(4) Atkinson, R (1989) Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds. J Phys Chem Ref Data Monograph No. 1. NY: Amer Inst Physics & Amer Chem Soc.

(5) Atkinson, R and Carter, WPL (1984) Kinetics and mechanisms of the gas-phase reactions of ozone with organic compounds under atmospheric conditions. Chem Rev 84: 437-470.

(6) Bevan C, Stadler JC, Elliott GS, Frame SR, Baldwin JK, Heung H-W, Moran E and Panepinto AS (1996) Subchronic toxicity of 4-vinylcyclohexene in rats and mice by inhalation exposure. Fundamental and Applied Toxicology, 32, 1-10.

(7) Calamari, D, Galassi, S, Setti, F and Vighi, M (1983) Toxicity of selected chlorobenzenes to aquatic organisms. Chemosphere 12, 253-262.

(8) Canadian Environmental Modelling Center (2004) Level I: Fugacity-Based Environmental Equilibrium Partitioning Model, Version 3.00. Canadian Environmental Modelling Centre, Trent University, Petersborough, Canada.

(9) Chemical Manufacturers Association (CMA) (1990) Report on the survey of the Butadiene Panel of the Chemical Manufacturers Association on 4-vinylcyclohexene. Submitted to the U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, D.C. May 3, 1990.

(10) Chemical Manufacturers Association (CMA) (1991) Industrial hygiene sampling for 4-vinylcyclohexene in the workplace - final report. Submitted to the U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, D.C. U.S. EPA/OPTS Public Files, Fiche #: OTS0533179. Doc#: 40-91109046. October 1, 1991.

(11) Chemical Products Safety Division, Basic Industries Bureau, Ministry of International Trade and Industry, Chemicals Inspection and Testing Instutute (1992). Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. Japan Chemical Industry Ecology-Toxicology and Information Center, Japan.

(12) Clark, B, Henry, JG and Mackay D. (1995) Fugacity analysis and model of organic chemical fate in a sewage treatment plant. Environ Sci Technol 29, 1488-1494.

(13) Cocheo, V and Bellomo, ML (1983) Rubber manufacture: sampling and identification of volatile pollutants. Am Ind Hyg Assoc J. 44, 521-527.

(14) Collins, JJ and Manus, AG (1987) Toxicological evaluation of 4-vinylcyclohexene. I. Prechronic (14-day) and subchronic (13-week) gavage studies in Fischer 344 rats and B6C3F1 mice. J Toxicol Environ Hlth, 21, 493-505.

(15) Collins, JJ, Montali, RJ and Manus, AG (1987) Toxicological evaluation of 4-vinylcyclohexene. II. Induction of ovarian tumors in female B6C3F1 mice by chronic oral administration of 4-vinylcyclohexene. J Toxicol Environ Hlth, 21, 507-524.

(16) Daubert, TE and Danner, RP. (1994) Physical and Thermodynamic Properties of Pure Chemicals Part 5. Taylor and Francis. (page number not available.)

(17) Degussa AG (undated) High Performance Building Blocks (brochure).

(18) Doerr, JK, Hooser, SB, Smith, BJ and Sipes, IG (1995) Ovarian toxicity of 4-vinylcyclohexene and related olefins in B6C3F1 mice: role of diepoxides. Chem Res Toxicol 8, 963-969.

(19) DuPont (1993) Two-week range-finding study with 4-vinylcyclohexene in rats and mice. Haskell Laboratory Report Number 759-793.

(20) DuPont (1994). Rat and mouse bone marrow micronucleus assay of 4-vinylcyclohexene following subchronic inhalation exposure, Revision No. 1. Haskell Laboratory Report No. 506-93. February 22, 1994. EPA Docket #OPTS-42116.

(21) EPI Suite (2004) Estimation Program Interface for Windows Suite. Version 3.12. Office of Pollution Prevention and Toxics and Syracuse Research Corporation, United States Environmental Protection Agency, Washington, DC, USA.

(22) Galassi, S and Vighi, M (1981) Testing toxicity of volatile substances with algae. Chemosphere 10, 1123-1126.

(23) Giannarini, C, Citti, L, Gervasi, PG and Turchi, G (1981) Effects of 4-vinylcyclohexene and its main oxirane metabolite on mouse hepatic microsomal enzymes and glutathione levels. Toxicol Lett 8, 115-121.

(24) Grizzle, TB, George, JD, Fail, PA, et. al. (1994) Reproductive effects of 4-vinylcyclohexene in Swiss Mice assessed by a continuous breeding protocol. Fundam Appl Toxicol 22, 122-129.

(25) Hermans, J, Canton, H, Janssen, P and De Jong, R (1984) Quantitative structure-activity relationships and toxicity studies of mixtures of chemicals with anaesthetic potency: acute lethal and sublethal toxicity to Daphnia magna. Aquatic Toxicology 5, 143-154.

(26) Hodgson, AT, Wooley, JD, and Daisey, JM. (1993) Emissions of volatile organic compounds from new carpets measured in a large-scale environmental chamber. Journal of the Air and Waste Management Association, 43, 316-324.

(27) Hooser, SB, Parola, LR, Van Ert, MD and Sipes IG (1993). Short Communication: Differential ovotoxicity of 4-vinylcyclohexene and its analog, 4-phenylcyclohexene. Toxicol Appl Pharm 119, 302-305.

(28) Howard, PH, Boethling, RS, Stiteler, WM, Meylan, WM, Hueber, AE, Beauman, JA and Larosche, ME (1992) Predictive model for aerobic biodegradability developed from a file of evaluated biodegradation data. Environ Toxicol Chem 11, 593-603.

(29) International Agency for Research on Cancer (1994) IARC monographs on the evaluation of carcinogenic risks to humans: 4-vinylcyclohexene. Vol 60, pages 347-359.

(30) International Agency for Research on Cancer (1994) IARC monographs on the evaluation of carcinogenic risks to humans: 4-vinylcyclohexene. Vol 60, pages 347; 354-355; 361.

(31) INVISTA, Inc. (2005) Vinylcyclohexene (VCH) Product Technical Data Sheet. INVISTA (TM) Specialty Intermediates.

(32) Keller, DA (1993) Partition coefficients of 4-vinyl cyclohexene and metabolites. Unpublished report, Haskell Laboratory Report No. 102-93 for Chemical Manufacturers Association, Washington DC, March 1993.

(33) Keller, DA, Carpenter, SC, Cagen, SZ and Reitman, FA (1997) In vitro metabolism of 4-vinylcyclohexene in rat and mouse liver, lung and ovary. Toxicol Appl Pharmacol 144, 36-44.

(34) Leifer, A (1993) Determination of rates of reaction in the gas-phase in the troposphere: theory and practice. 5. Rate of indirect photoreaction. EPA/744/R-93/001 (NTIS PB93-149334). U.S. Environmental Protection Agency, OPPT, Washington, DC.

(35) Lide, DR (ed.) (2004) CRC Handbook of Chemistry and Physics. 85th Edition. CRC Press LLC Boca Raton, FL p. 3-568.

(36) Mackay, D et al. (1996) Evaluating the environmental fate of a variety of types of chemicals using the EQC model. Environ Toxicol Chem 15, 1627-1637.

(37) Mackay, D. et al. (1996) Assessing the fate of new and existing chemicals: a five-stage process. Environ Toxicol Chem 15, 1618-1626.

(38) Meylan, W, Howard, PH and Boethling, RS (1992) Molecular topology/fragment contribution method for predicting soil sorption coefficients. Environ Sci Technol 26, 1560-1567.

(39) Meylan, WM and Howard, PH (1993) Computer estimation of the atmospheric gas-phase reaction rate of organic compounds with hydroxyl radicals and ozone. Chemosphere 26, 2293-99.

(40) Meylan, WM, Howard, PH, Boethling, RS et al. (1999) Improved method for estimating bioconcentration / bioaccumulation factor from octanol/water partition coefficient. Environ Toxicol Chem 18, 664-672.

(41) Mill, T, Haag, W, Penwell, P, Pettit, T and Johnson, H (1987) Environmental fate and exposure studies development of a PC-SAR for hydrolysis: esters, alkyl halides and epoxides. EPA Contract No. 68-02-4254. Menlo Park, CA: SRI International.

(42) Ministry of Environment (2000). 4-Vinylcyclohexene ecotox data cited in the National Institute of Technology and Evaluation database. Tokyo, Japan.

(43) MITI (Ministry of International Trade and Industry) (1992). Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. CITI ed. (Chemicals Inspection & Testing Institute). Published by Japan Chemical Industry Ecology-Toxicology & Information Center.

(44) Mount, GH and Eisele, FL (1992) An intercomparison of tropospheric OH measurements at Fritz Peak Observatory, Colorado. Science 256, 1187-8.

(45) Neuhauser, EF, Durkin, PR, Malecki, MR, and Anatra, M (1986) Comparative toxicity of ten organic chemicals to four earthworm species. Comp Biochem Physiol 83C, 197-200.

(46) Neuhauser, EF, Loehr, RC, Malecki, MR, Milligan, DL, and Durkin, PR (1985) The toxicity of selected organic chemicals to the earthworm Eisenia fetida. Journal of Environmental Quality 14, 383-388.

(47) NTP (1981) National Toxicology Program Database Search Application.
http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm.
CAS#100-40-3. Genetic Toxicity Studies. Salmonella. Study No. 777152. Detailed Study Data.

(48) NTP (1984) National Toxicology Program Database Search Application.
http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm.
CAS#100-40-3. Genetic Toxicity Studies. CHO Cell Cytogenetics-Chromosome Abberations. Study No. 169960. Detailed Study Data.

(49) NTP (1984) National Toxicology Program Database Search Application.
http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm.
CAS#100-40-3. Genetic Toxicity Studies. CHO Cell Cytogenetics-Sister Chromatid Exchange. Study No. 169960. Detailed Study Data.

(50) NTP (1986) Toxicology and carcinogenesis studies of 4-vinylcyclohexene (CAS No. 100-40-3) in F344/N rats and B6C3F1 mice (gavage studies). NTP Technical Report 303, NIH Publication No. 86-2559, August 1986.

(51) NTP (1989) National Toxicology Program Database Search Application.
http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm.
CAS#100-40-3. Genetic Toxicity Studies. Salmonella. Study No. 609542. Detailed Study Data.

(52) NTP (undated) National Toxicology Program Database Search Application.
http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm.
CAS#100-40-3. Genetic Toxicity Studies. Mouse Lymphoma Studies. Study No. 971117. Detailed Study Data.

(53) Rappaport, SM and Fraser, DA (1977) Air sampling and analysis in a rubber vulcanization area. Am Ind Hyg Assoc J, 38, 205-210.

(54) Sabljic, A (1984) Predictions of the nature and strength of soil sorption of organic pollutants by molecular topology. J Agric Food Chem 32, 243-246.

(55) Sabljic, A (1987) On the prediction of soil sorption coefficients of organic pollutants from molecular structure: application of molecular topology model. Environ Sci Technol 21, 358-366.

(56) Smith, BJ and Sipes IG (1991) Epoxidation of 4-vinylcyclohexene by human hepatic microsomes. Toxicol Appl Pharmacol 109, 367-371.

(57) Smith, BJ, Carter, DE and Sipes, GI (1990) Comparison of the disposition and in vitro metabolism of 4-vinyl cyclohexene in the female mouse and rat. Toxicol Appl Pharmacol 105, 364-371.

(58) Smith, BJ, Mattison, DR and Sipes, GI (1990) The role of epoxidation in 4-vinyl cyclohexene-induce ovarian toxicity. Toxicol Appl Pharmacol 105, 371-381.

(59) Smith, BJ, Sipes, IG, Stevens, JC and Halpert, JR (1990) The biochemical basis for the species difference in hepatic microsomal 4-vinylcyclohexene epoxidation between female mice and rats. Carcinogenesis 11, 1951-1957.

(60) Smyth, HF, Carpenter, CP, Weil, CS et al. (1962) Range finding toxicity data: List VI. Am Ind Hyg Assoc J, 23, 95-107.

(61) Smyth, HF, Carpenter, CP, Weil, CS et al. (1969) Range finding toxicity data: List VII. Am Ind Hyg Assoc J, 30, 470-476.

(62) United States Environmental Protection Agency (USEPA) (1989) Notice containing the ITC recommendation of 4-VCH to the Priority List and soliciting interested parties for developing a consent order for 4-VCH. 54 FR 51114. December 12, 1989.

(63) United States Environmental Protection Agency (USEPA) (1991) Fish chronic toxicity data base. Duluth, MN: Environmental Research Laboratory (ERL), Office of Research and Development, USEPA, 6201 Congdon Boulevard, 55804; contact C.L. Russom (218) 720-5500.

(64) United States Environmental Protection Agency (USEPA) (1991) OTS PMN ECOTOX. Washington, DC: USEPA, Office of Toxic Substances.

(65) USFDA (2002) Effective notifications for food contact substances. FCN No. 244. September 26, 2002.

(66) Van Duureen, BL, Nelson, N, Orris, L, et. al. (1963) Carcinogenicity of epoxides, lactones and peroxy compounds. Journal of the National Cancer Institute 31, 41-55.

(67) Veith, GD, Call, DJ, and Brooke, LT (1983) Structure-toxicity relationships for the fathead minnow, Pimephales promelas: narcotic industrial chemicals. Canadian Journal of Fisheries and Aquatic Sciences 40, 743-748.

(68) Watabe, T, Hiratsuka, A, Ozawa, N and Isobe, M (1981) A comparative study on the metabolism of d-limonene and 4-vinylcyclohex-1-ene by hepatic microsomes. Xenobiotica 11, 333-344.

(69) Wong, V and Wang, S-H (1996) Styrene from butadiene via 4-vinylcyclohexene by the Dow process. Process Economics Program Review (#94-2-4), SRI Consulting, January 1996.

(70) Yalkowsky, SH (2003) Aqueous Solubility Data. CRC Press LLC Boca Raton, FL p. 509.