Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

4-vinylcyclohexene (VCH)

CAS number: 100-40-3

EC number: 202-848-9

ECHA/RAC/ CLH-O-0000002966-62-01/A2

Adopted

14 September 2012

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

ECHA has compiled the comments received via internet that refer to several hazard classes and entered them under each of the relevant categories/headings as comprehensive as possible. Please note that some of the comments might occur under several headings when splitting the given information is not reasonable.

Substance name: 4 vinylcyclohexene (VCH)
EC number: ***202-848-9***
CAS number: ***100-40-3***

General comments

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
14/07/2011	United Kingdom / Member State Competent Authority	We believe that there is sufficient information in the dossier to enable the reader to come to conclusion on the classification for carcinogenicity.	Thanks for your support	Noted
30/06/2011	Germany / Member State	The German CA agrees with the proposed classifications. However, we suggest the following changes. P. 22, section 4.1.2, end of paragraph: The authors describe the possibility that the CYP activity could be modified by possible exposure to drugs or environmental chemicals. Since the authors described also the important role of CYP2E1 in the metabolism of VCH (see p. 23) it should be pointed out that CYP2E1 induction in human liver can easily be achieved by regular ethanol consumption. Regular and heavy drinker should be considered as population with increased potential to activate VCH to carcinogenic metabolites. P. 24, second paragraph, third line: Replace "metabolisation" by "metabolism".	Thanks for your support. Modifications proposed accepted.	Noted
29/06/2011	Spain Member State	We are wondering why the environmental classification proposal has not been included for this substance. We have found information to make a environmental classification proposal.	Cf rules detailed in Art.36 of the CLP regulation	Noted
05/07/2011	Netherlands / Bureau REACH / Member State	Administration information (not to be distributed, and disclosed) This may be a duplication of our comments, due to the holiday season we are unable to check this. General Comments: We agree that the ovarian neoplasms in female mice are the most pronounced carcinogenic effects of VCH. However, despite the high mortality among male and female rats (low and high dose groups) and male mice (high dose group only), several treatment-related tumours were observed in these groups, which does provide additional	Thanks for your support. We acknowledge that the incidences of different type of tumors were increased in male and female rats and	In RACs view there is a distinct difference between the clear findings of ovary tumours in low dosed female mice, where the mortality was

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		signs of a carcinogenic potential. Therefore, these data should not simply be ignored. We agree that the ovarian tumours in female mice are possibly induced by the metabolisation of VCH into VCD (supported by the data that VCD does also induce ovarian tumours in mice and rats), which occurs in mice at a much higher rate than in rats. This mechanism indeed explains the absence of ovarian tumours in rats. Since human hepatocytes have been shown to be able to metabolise VCH into VCD, there is no evidence that this mechanism is not relevant to humans. Therefore, we agree with the proposed classification for carcinogenicity: Carc. 1B; H 350 (CLP) or Carc. Cat. 2; R45 (DSD).	in male mice. However, due to the poor survival rate in these groups, the interpretation of these tumors in those treated groups could be misleading. Early excessive mortality may have masked the higher outcome of tumors induced by VCH. Nevertheless, the increased incidence of adenomas or squamous-cell carcinomas (combined) of the clitoral gland in low-dose female rats has to be taken into account since the survival of these animals is similar to control until week 102.	comparable to the control group, and other findings. Apart from the clear finding of ovary tumours in mice, there were some minor findings of other tumours in mice and rats. These findings indicated carcinogenic potential of VCH in e.g. skin, adrenal gland, anterior pituitary gland and in the clitoral gland of rats, and in the lungs of mice. Because of the high mortality in these dose groups, the reliability of these studies was compromised, thereby not providing adequate evidence to draw conclusions.
06/07/2011	United States / Individual	I am Dr. Christopher Bevan, PhD, DABT, a toxicology consultant and managing principal of CJB Consulting LLC. The CLH report did not include or evaluate the substantial number of peer-reviewed publications which provide important information on the mode-of-action (MOA) of the ovarian tumors seen in mice from VCH exposure. In ECHA's Guidance on the Application of the CLP Criteria for classification for carcinogenicity, it states on page 309 that "all available data must be considered carefully to judge if it can be concluded with confidence that the tumours are being induced through a specific mechanism." This was not done for VCH in the CLH report. The	The destruction of oocytes, which is likely a critical step in the induction of ovary carcinogenesis induced by VCH or VCD, has been	We thank the MSCA for providing more information on the Mode of Action (MoA) after the public consultation. We agree with the

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		data indicate that ovarian tumors in mice occur as a result of a cytotoxic effect (oocyte depletion by apoptosis in the ovary) caused by a metabolite of VCH (VCH diepoxide), followed by a hormonal response on the ovary. The lack of a genotoxic response by VCH when tested in both in vitro and in vivo genotoxicity assays further support the conclusion that VCH acts as a non-linear (threshold) carcinogen in mice. Although the MOA is relevant to humans, it implies, however, that there is a practical threshold above a certain dose level, and if VCH was to be classified as a carcinogen, a Category 2 classification would be more appropriate.	reported in the sections 4.10.4 Summary and discussion of carcinogenicity and 4.10.5 Comparison with criteria. The CLH report has been revised to provide more information on this MOA, based on your communication and on the review from Hoyer and Sipes (2007). With regard to the genotoxicity potential of VCH, Paragraph 4.9.4 of our proposal explains why the mutagenic potency of VCH (and also VCD) is not so clear to us (see also our response to comment below from Germany / AffiliatedWith Organisation / Company-Manufacturer). We consider that uncertainty remains regarding the	MSCA that uncertainty remains regarding the mutagenic potential of VCH, but there is low concern based on available information.

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			mutagenic potential of VCH. We did not propose a classification as data are lacking but this effect cannot be excluded and this endpoint is foreseen for substance evaluation. Therefore, this argument should not impact the category of classification.	
14/07/2011	Germany / AffiliatedWithOrganisation / Company-Manufacturer	Please find attached our comments on Annex XV dossiers proposing harmonised Classification & Labelling for substance CAS 100-40-3. *ECHA comment: The document attached "Comment to the French proposal for Harmonized Classification and Labeling of 4-Vinylcyclohexene (CAS 100-40-3)" is copied below:* Comment to the French proposal for Harmonized Classification and Labeling of 4-Vinylcyclohexene (CAS 100-40-3) The CLH dossier of 4-Vinylcyclohexene (VCH) which was provided to EChA by France suggests a classification for carcinogenicity in category 1B in accordance with the CLP regulation (EC) 1272/2008. However, based on the available data the criteria for Carc. Cat. 1B are clearly not met and classification in Carc. Cat. 2 is proposed at worst due to the following facts: a) Scientific discussion 1) Metabolism of VHC (addition to "Summary and discussion on toxicokinetics" of the French CLH report, chapter 4.1.3, pp 22 -24) Metabolism of VCH is described in Figure 1 (taken from Keller et al. 1997). VCH is converted to VCD in a two step process. VCD is supposed to be the active metabolite causing ovarian	The critical point of the classification of VCH as Carc Cat. 1B is the human relevance of the VCH-induced ovary tumors in mice. This assumption is questioned on the basis of the results of the study of Smith and Sipes (1991) which determined the rate of formation of VCH 1,2 epoxide in human liver microsomes to be 13- and 2-fold less than in mouse and	We agree with the MSCA that the metabolism of VCH into monoepoxide and then into the diepoxide VCD, and consequently the VCH-induced ovotoxicity and ovary carcinogenesis could be relevant to humans. We agree with the MSCA that no firm conclusion can be drawn about the genotoxic potential of VCH, however based on available

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		toxicity in rat and mice and ovarian carcinogenicity in mice. No ovarian carcinogenicity was found in rats. The epoxidation of VCH to VCH-1,2-epoxide is the velocity determining step and therefore the critical reaction for ovarian toxicity and carcinogenicity. The epoxidation of VCH to VCH-1,2-epoxide shows distinct differences between mice, rats and humans (Table 1). Formation of relevant amounts of the toxic metabolite VCD is rather mouse specific. In-vitro studies have proven VCD formation directly from VCH in mice but failed to show VCD formation directly from VHC for rat and human supersomes (Fontaine et al. 2001). Existing studies suggest that rats are the appropriate animal model for extrapolation of animal data to humans. This conclusion was also published by the same researchers in a recent comprehensive review concerning the toxicity of VCH (Hoyer, 2007). Details: Rajapaksa and coworkers (2007) evaluated the role of ovarian CYP2E1 in VCH-induced ovarian toxicity showing that despite in vitro ovarian bio-activation of VCH or VCH-1,2-epoxide requires CYP2E1 enzyme, in vivo CYP2E1 plays a minimal role. It was concluded in the French CLH Dossier that these findings support that hepatic metabolism dominates bioactivation of VCH and VCH-1,2- epoxide to the ovarian toxic metabolite, VCD. Therefore, the consecutively described data are focusing on the well examined liver metabolism. Keller and co-workers investigated the in vitro metabolism of VCH in microsomes of rats and mice (Keller et al., 1997). It was shown that mouse liver had a Vmax for the generation of VCH-1,2-epoxide from VCH that was 56-fold higher than that for rat liver. Rat and mouse liver had very similar Km and Vmax values for the metabolism of vinylcyclohexene-1,2-epoxide to VCD indicating no species difference for this step. Hydrolysis of VCD was detected in rat and mouse liver and lung as well as in rat ovary microsomes. The Vmax for rat liver was 9-fold greater than that for mouse liver. Smith and Sipes found that the rate of the formation of 4-vinylcyclohexene-1,2-epoxide from VCH in hepatic microsomes obtained from humans was 13- and 2-fold lower than that from mouse (B6C3F1) and rat (F344), respectively (Smith and Sipes, 1991). It has been shown in "Supersomes" containing purified human CYP and purified human P450 reductase as well as cytochrome b5 and other cofactors in excess that VCH mono epoxide from VCH is formed and in another experiment formation of VCH diepoxide from VCH mono epoxide was shown in this really artificial system. (Fontaine et al 2001b). Therefore, formation of VCD from VCH is theoretically possible. However, no direct formation of VCH di epoxide from VCH was shown in this test system (Fontaine et al 2001a).	rat hepatocyte microsomes (results from a previous study (Smith *et al.*, 1990a), respectively. However, published literature demonstrates variability in the rate of formation of VCH epoxides. Indeed, it appears that the difference in the rate of formation of VCH monoepoxides (i.e. the critical step thought to account for the higher mouse sensitivity towards VCH-induced ovotoxicity) in rat liver microsomes compared to mouse liver microsomes varies from study to study (from a factor of 1.9 to 55.5 for VCH 1,2 epoxide, and from 1.6 to 13 for VCH 7,8 epoxide (Fontaine *et al.*, 2011a; Keller *et al.*, 1997; Smith *et*	data concern is low contrary to the documented genotoxicity of VCD. The strength of evidence of finding of the ovary tumours in mice is good. However the high mortality in the other groups hampers the strength of these findings and after a weight-of-evidence analysis we disagree with the MSCA that Carc Cat 1B is appropriate for VCH. In our opinion this is a borderline case between category Carc. 1B and 2. However, based on all available information RAC regards this as a category 2 (CLP) carcinogen. A minor editorial error in the response from MSCA is highlighted. The correct reference is

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		In conclusion, the balance of activation vs. detoxification reactions in rats and mice suggests that the mouse may be more susceptible to 4-VCH toxicity because of generation of high levels of epoxide metabolites. In general, the mouse is more efficient at metabolism of 4-VCH to epoxides than is the rat or human. Beyond this, the rat seems to be more efficient at hydrolysis of epoxides. Thus, the rat would tend to produce a lower concentration of epoxide metabolites than the mouse, at equal doses of 4-VCH. This has been also demonstrated in vivo. After an i.p. administration of VCH (800 mg/kg bw), VCH-1,2-epoxide (41 nmol/mL) was found in blood of mice, but was not detected in rats (Smith et al 1990). This is also supported by the fact that VCD could be detected after incubation of hepatic microsomes from mice with VCH. Hepatic microsomes from rats treated in the same way showed no detectable VCD formation from VCH (Fontaine et al., 2001a). Same result is true for human CYP "Supersomes" (human CYP + P450 reductase + cytochrome b5). Supersomes are not able to directly catalyze VCH epoxidation to diepoxide metabolites of VCH in detectable amounts (Fontaine et al., 2001a). This demonstrates VCD is practically not available to the rat and human after exposure to 4-VCH. The differences in the metabolism of VCH by the rat and mouse explain why after administration of VCH higher internal exposure to active epoxides occurs in mice (Smith et al., 1990a + b). This has also been suggested as the reason for the different sensitivities of the rat and mouse with regard to the ovarian toxicity and carcinogenic effects of VCH (Hoyer & Sipes, 1996). The rate of hepatic VCH epoxidation can be regarded as the main factor which determines the ovotoxicity and carcinogenicity of VCH. It has been demonstrated in in-vitro studies that humans are less capable forming the VCH-1,2-epoxide than the F344 rat and therefore be even regarded as less sensitive than the F344 rat to VCH-1,2 epoxide transmitted toxic effects. But taking other rat strains into account one can conclude that VCH-1,2-epoxide formation of rat and humans are about the same. The results of the studies above suggest that rats are the more appropriate animal model for extrapolation of animal data to humans. Formation of relevant amounts of the toxic metabolite VCD is rather mouse specific. VCD is practically not available to the rat and human after exposure to 4-VCH. Therefore, ovarian carcinogenicity was observed in mice but not in rats.	*al*., 1990a). Taking into account the results from the study of Fontaine *et al*. (2001a), the mean rate of formation of VCH-1,2-epoxide in human liver microsomes is only 1.3-fold lower than in mice and even 1.4-fold higher than in rats (when comparing the highest rate of VCH-1,2-epoxide formation in women, it is even 1.4 and 2.7 higher than in mice and rats, respectively). Moreover, induction of VCH epoxidation has been demonstrated in both rats and mice. Indeed, increased formation of monoepoxides from 2 to 4-fold were observed in both rats and mice after a previous exposure to VCH for 10 days (Fontaine *et al*.,	Fontaine et al., 2001a.

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		(Table 1 should be added to chapter 4.1.1 "toxicokinetics: non human information" of the French CLH report (pp 17 -22) for clarity) Table 1: Maximal conversion velocity (nmol/min/mg/protein) of 4-VCH and selected metabolites of VCH in rat, mice and humans ([K]Keller et al. 1997; [S]Smith and Sipes 1991; [W]Watabe et al. 1991)	2001a). In addition, the study of Smith and Sipes clearly demonstrated that human hepatocytes microsomes are able to produce VCH-1,2- and -7,8-epoxide. Fontaine *et al*. showed that isolated CYPs were capable of significantly converting VCH into VCH monoepoxide, and then monoepoxides into VCD. VCD could not be directly produced at detectable levels by "supersomes" incubated with VCH (Fontaine *et al*., 2001b). Nevertheless, it should be noted that, similarly, VCD was not detected in mouse or rat microsomes incubated with VCH when rodents were not previously exposed to VCH, indicating that an induction of	

Conversion	**Liver**			**Lung**		**Ovar**	
	Mouse (BeC3F1)	**Rat (Strain)**	**Human (n =12)**	**Mouse**	**Rat**	**Mouse**	**Rat**
4-VCH to 4 VCH-1,2 epoxide	9.1[S] 11.1[K] Fontaine 2001: 0.9	1.4[S] (F344) 0.49[W] (Wistar) 0.20[K] (Crl:CD BR) Fontaine 2001: 0.47	0.67[S] M 0.23-0.85 (n=6) F 0.36-1.25 (n=5)	3.49	1.39	Not detectable	Not detectable
4 VCH to 4 VCH-7,8 epoxide	0.91[K] Fontaine 2001: 0.61	0.12[S] (Wistar) 0.07 [K] (Crl:CD BR) Fontaine 2001 (F344): 0.37	<0.09[S]	1.83	Not detectable	Not detectable	Not detectable
4 VCH-1,2 epoxide to VCD	5.35	3.69	Not examined*	2.70	2.06	Not detectable	Not detectable
4 VCH-7,8 epoxide To VCD	9.45	8.83[K]	Not examined*	11.8	1.35	Not detectable	Not detectable

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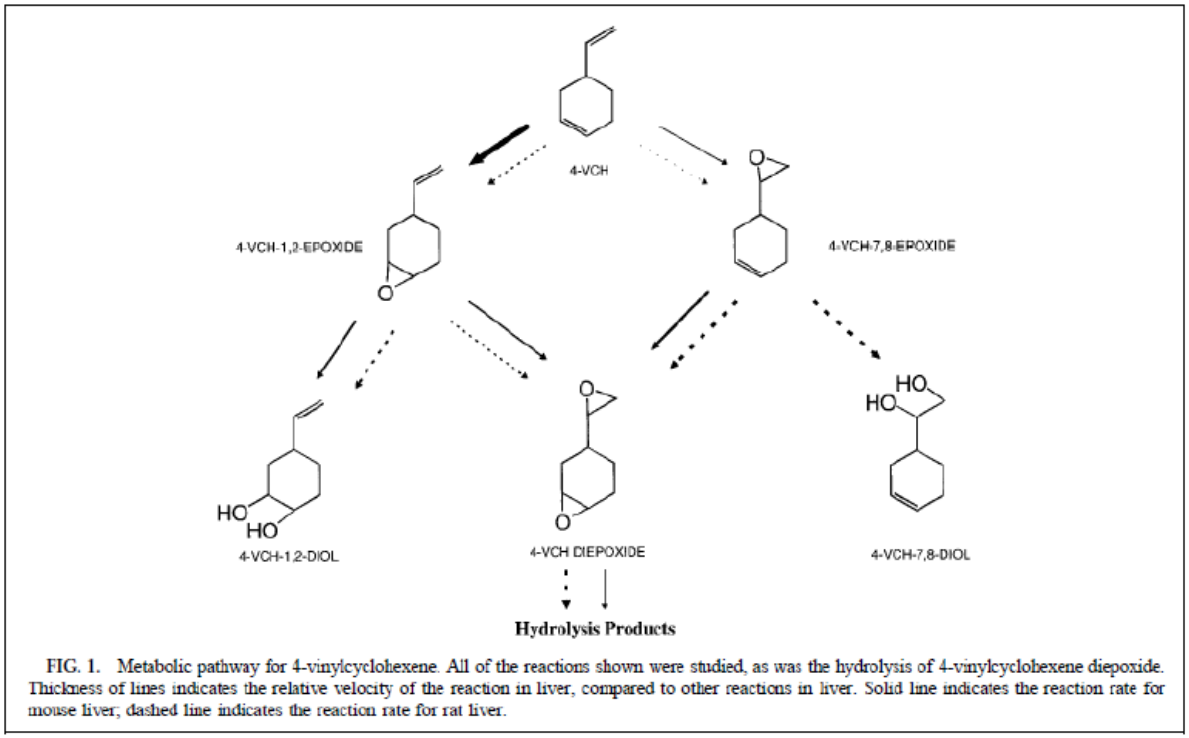
		* it was shown by Fontaine et al. (2001, 2000) that artificial human "supersomes" are capable to form VCH mono epoxide from VCH and VCD in relevant amounts only from VCH mono epoxide as the rat.  FIG. 1. Metabolic pathway for 4-vinylcyclohexene. All of the reactions shown were studied, as was the hydrolysis of 4-vinylcyclohexene diepoxide. Thickness of lines indicates the relative velocity of the reaction in liver, compared to other reactions in liver. Solid line indicates the reaction rate for mouse liver; dashed line indicates the reaction rate for rat liver. Figure 1: Metabolic pathway of 4-VCH taken from Keller et al. 1997, French CLH report, Respectively 2) VCH mutagenicity (addition to chapter 4.9.4 "summary and discussion of mutagenicity, 4.9.5 "Comparison with criteria" and 4.9.6 "Conclusion on classification and labeling" of the French CLH report) VCH is clearly non-mutagenic in in-vitro and in vivo OECD Guideline studies. VCH did not produce an increase of revertants in TA1537 with or without metabolic activation (rat or hamster S9). In contrast, VCD was positive in the Ames test for TA1537 with metabolic	specific CYPs is required (Fontaine et al., 2001a). In addition, the results of the Fontaine's study could demonstrate that a combination of different human isozymes is likely needed to convert VCH into VCD. Overall, there is evidence that human CYPs are able to catalyze the different enzymatic reactions leading to the formation of the ultimate metabolite, VCD. There is no evidence of clear species differences for the capability of metabolising VCH into VCD. Therefore, we are not convinced that the metabolism of VCH into monoepoxide and then in the diepoxide VCD, and consequently the VCH-induced ovotoxicity and	

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activation. Equivocal results were obtained for TA 1537 without metabolic activation (NTP, 1989).

It is known that S9 mix is containing microsomes and enriched with necessary enzymes for VCD generation (eg CYP2A and CYP 2B).

Details
VCH did not produce an increase of revertants in the strains TA100, TA1535, TA1537, and TA98, with or without metabolic activation (rat or hamster S9), when tested according to the pre-incubation protocol (NTP 1986).
VCH did not induce micronuclei in OECD 474 in vivo studies with rats and mice after oral and inhalative administration (Bevan, 2001). Signs of toxicity and decreased body weight gain were observed in treated rats. Body weight gain was decreased in high-dose male mice in the 2-day study and increased mortality was observed in the high-dose groups in the 13-week study.

In contrast to this VCD was positive in the Ames test for TA1537 with metabolic activation. Equivocal results were obtained for TA 1537 without metabolic activation (NTP, 1989).
There is no in-vivo data available for VDC.

3) Discussion of Carcinogenicity
(Table 2 an 3 should be added to chapter 4.10.1.1 "toxicokinetics: non human information" of the French CLH report, p41 for clarity)
Table 2: Carcinogenicity of VCH in rats after 2 years oral dosing (gavage) of 0, 200 or 400 mg/kg/day (NTP 1986)

<table>
<tr><td>Dose</td><td></td><td>0</td><td>200</td><td>400</td><td>Historical control % (range)</td><td>Comment</td></tr>
<tr><td>Survival (104 W)</td><td>M
f</td><td>33/50
40/50</td><td>13/50
28/50</td><td>5/50
13/50</td><td></td><td></td></tr>
<tr><td>Squamous Cell Papilloma or Carcinoma</td><td>m</td><td>0/50</td><td>1/50 (2%)</td><td>4/50 (8%)</td><td>1.9 (0-10)</td><td>No effect as within range of historical control</td></tr>
<tr><td>Preputial</td><td>m</td><td>1/50</td><td>1/50</td><td>3/50</td><td>3.6 (max 14)</td><td>No effect as</td></tr>
</table>
</td><td>ovary carcinogenesis are not relevant to human.

Another critical point of the dossier is the genotoxic potential of VCH. As mentioned in the CLH dossier (4.9.4 Summary and discussion of mutagenicity), in vitro systems may be inappropriate to test VCH since rat S9 may fail to metabolise VCH into the ultimate metabolite VCD. Interestingly, a mouse lymphoma assay was found positive in a NTP study (not published but results available on the NTP website (http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=mouselymphoma.studyDetails&study_no=971117&cas_no=100-40-3&endpointlist=M</td><td></td></tr>
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Gland: Adenoma or Carcinoma						within range of historical control
Adenoma or carcinoma Anterior Pituitary Gland	F	19/50 (38%)	24/48 (50%)	7/44 (16%)	41.3 (27-60)	No effect
Clitoral Gland: Adenoma or Squamous Cell Carcinoma	F	1/50 (2%)	5/50 (10%)	0/49	2.1 (0-8)	No effect as within range of historical control and no dose dependency

Table 3: Carcinogenicity of VCH in mice after 2 years oral dosing (gavage) of 0, 200 or 400 mg/kg/day (NTP 1986)

dose		0	200	400	Historical control % (range)	Comment
Survival (104 W)	m f	37/50 40/50	39/50 39/50	7/50 17/50		
Alveolar/Bronchiolar Adenoma or Carcinoma	m	5/50 (8%)	11/50 (22%)	4/50 (8%)	14.3 (2-26)	No effect as within range of historical control and no dose dependency
Adrenal gland adenoma	m	0/50	3/49 (6%)	4/48 (8%)	0.7 (0-4.3)	No effect as within range of historical control
Mixed ovarian Tumor, (Benign)	F	0/49 (0%)	25/48 (52%)	11/47 (23%)	0.2%	effect

L,ML-N)). In vivo genotoxicity was investigated by a micronucleus assay in rats and mice exposed to VCH by inhalation for 2 days or 13 weeks (Bevan *et al.*, 2001). However, validity of the results is questioned since only 1000 PCE per animal were scored (the actual OECD 474 TG recommends to score a minimum of 2000 immature erythrocytes per animal for the incidence of micronucleated immature erythrocytes), no individual data are available, and no historical negative/positive control data are available (especially for 1,3-butadiene used as a positive control in the mouse micronucleus assay)). With

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Ovarian Granulosa Cell adenoma	F	1/49 (2%)	9/48 (19%)	11/47 (23%)	Close to 0 % (unusual tumor)	effect

The following text should be added to chapter 4.10.4 "summary and discussion of carcinogenicity" of the French CLH report (pp.43-45):
Carcinogenic studies were performed with mice and rats (NTP, 1986; Collins, 1987). High mortality was observed in high dose rats and mice (survival: 3/50 (10%) male rats; 5/50 (14%) male mice; 13/50 (26%) female rats; 17/50 (34%) female mice (Table 2 and 3)). In general, male animals are more sensitive to VCH than female animals and rats are more sensitive than mice. High dose results are questionable for rat and mice due to high morality. Additionally, low dose results are questionable for male rat as well. Reliability of the study may be considered as not assignable to invalid as only results of low dose male and female mice and low dose female rats can be discussed.
Results of the NTP study are questionable and can provide only limited evidence therefore. Nevertheless, as these studies are the only available carcinogenicity studies they are briefly discussed.
No carcinogenic effect in comparison to the control was observed in female rats receiving a dose of 200 mg/kg/day for 2 years (Table 1). Tumors observed are either in the range of the control (data not shown) or within the range of the historical control (table 2). High dose male and female data as well as low dose male data is presented, but not taken into depth consideration.

Ovarian carcinogenicity was observed in mice. This kind of tumor is unusual (see historical control). Other tumors observed are either in the range of the control (data not shown) or within the range of the historical control (table 3).

The toxicokinetic studies mentioned before suggest that rats are the more appropriate animal model for extrapolation of animal data to humans. Formation of relevant amounts of the toxic metabolite VCD is rather mouse specific.

In general, the mouse is more efficient at metabolism of 4-VCH to epoxides than is the rat or human. Beyond this, the rat seems to be more efficient at hydrolysis of epoxides. Thus, the rat would tend to produce a lower concentration of epoxide metabolites than the mouse, at equal doses of 4-VCH. The balance of activation vs. detoxification reactions in rats and mice suggests that the mouse may be more susceptible to 4-VCH toxicity.

regard to VCD, no in vivo assay is available, although it is mutagenic in several in vitro tests and carcinogenic in rats and mice. However, it was found to form DNA adducts in mice dermally exposed to VCH (Randerath and Mabon, 1996). Therefore, we are of the opinion that no firm conclusion can be drawn about the genotoxic potential of VCH and VCD and that this endpoint has not been sufficiently investigated.

Finally, concern has been raised about a classification as Carc Cat 1B based on the increased incidence of ovary tumors in female mice only. The CLP states: *"A single study in one*

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		In-vitro studies have proven VCD formation directly from VCH in microsomes from mice but failed to show VCD formation directly from VHC for rat microsomes and artificial produced human supersomes (Fontaine et al. 2001). Therefore, rats are the appropriate animal model for extrapolation of animal data to humans. VCD can be regarded as the ultimate carcinogen in mice. However, VCD is practically not available to rats and humans after exposure to 4-VCH. Therefore, ovarian carcinogenicity was observed in mice, but not in rats. As rats are considered the appropriate animal model for extrapolation of VCH animal data to humans (Hoyer, 2007), the relevance of the ovarian carcinogenicity for humans remains unclear or is unlikely. 3) Missing epidemiological evidence There is no epidemiological study available to evaluate the carcinogenicity of VCH to humans. There is limited evidence for the carcinogenicity of VCH to experimental animals. B) Comparison with Classification Criteria The chapter "Comparison with criteria for classification" of the French CLH report (pp45 – 46) has to be changed. Bases on the comment given above assumption made in chapter 4.10.5 are not correct or incomplete, respectively. In compliance with Regulation (EC) No 1272/2008 (CLP), substances are classified for carcinogenicity according to their potential to cause cancer in humans. The classification criteria for cancer classification are given below and compared to the available data for VCH. Criteria to be considered for classification Cancer Cat 1 <u>*Cancer Cat. 1: Known or presumed human carcinogens*</u> *A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:* <u>*Category 1A, known to have carcinogenic potential for humans*</u>*,* *classification is largely based on human evidence, or* <u>*Category 1B, presumed to have carcinogenic potential for humans*</u>*,* *classification is largely based on animal evidence.* *The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2).* *Such evidence may be derived from:*	*species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites"*. Granulosa-cell tumors, an uncommon finding in NTP historical vehicle control, were observed in low-dose and high-dose female mice. Although the mortality was significantly increased in the high-dose group, the data should not be disregarded because they are also found in the low-dose group. In addition, VCD produced the same type of tumors in mice (NTP, 1986;	

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		– human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or *– animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen).* *In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.* Additional criteria to be considered for classification: ***Section 3.6.2.2.3 (CLP):*** *Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the substance and an increased incidence of tumours. Limited evidence in humans is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. The terms 'sufficient' and 'limited' have been used here as they have been defined by the International Agency for Research on Cancer (IARC) and read as follows:* *- **sufficient evidence** of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;* *- limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.*	1989). Not only benign tumors were observed in treated female mice but also carcinoma. It should be noted that the authors of the NTP study reported that the granulosa-cell lesions are a continuum of hyperplastic to benign and malignant neoplastic proliferations. It should be emphasised that increased incidence of tumors have been observed in male mice and male and female rats, but, due to poor survival in these groups, the interpretation of the data is difficult. Excessive mortality may have masked increased incidences of different types of tumors. Also, we do not think that ovary tumors observed in mice	

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		According to CLP section 3.6.2.2.6, important factors which may be taken into consideration when assessing the overall level of concern for humans are: *(a) tumour type and background incidence;* *(b) multi-site responses;* *(c) progression of lesions to malignancy;* *(d) reduced tumour latency;* *(e) whether responses are in single or both sexes;* *(f) whether responses are in a single species or several species;* *(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;* *(h) routes of exposure;* *(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;* *(j) the possibility of a confounding effect of excessive toxicity at test doses;* *(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.* Rational for non-classification of VCH into Carc. Cat. 1A/B VCH does not meet the criteria for Carc. Cat. 1A as there is no valid epidemiological data available that justifies a cancer classification. VCH does not met the criteria for Carc. Cat 1B due to the following reasons: 1. There are no human studies that give even limited evidence for a causal relation ship of carcinogenicity and VCH exposure. 2. Detailed toxicokinetic data suggests that mechanism causing ovarian tumor is rather mice specific and therefore not relevant to rats or humans (see consideration criteria (i)). Only mice, but not rats or humans are able to metabolize VCH to VCD in a significant amount (steady state concentration). VCD is considered to be a carcinogen (Carc. Cat. 2 but not 1B according to regulation 1272/2008) 3. Significant tumor were only observed in mice (see consideration criteria (f) – reason see 2.). No tumor are observed in rats. 4. Predominantly benign ovarian tumor were observed in mice and ovarian tumors were the only site tumor induction was observed (no multisite response, see consideration criteria (b) – reason see 2.)	could be confounded with "excessive toxicity at test doses" since the incidence is statistically significant even at the low dose at which mortality was similar to control. Overall, we are still convinced that VCH shows sufficient evidence of carcinogenic potential. Based on the fact that VCH is clearly carcinogenic in female mice, that there is uncertainty regarding the genotoxicity of VCH and VCD, and that there is no evidence that the VCH-induced ovary carcinogenesis is not relevant to humans, a classification in Carc Cat 1B is still appropriate for VCH. With regard to	

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		5. High dose groups of existing animal studies in rat and mice are inadequate for evaluation as survival rate is far to low (10-34%). Tumor findings in this studies may therefore be not regarded of sufficient evidence but of limited evidence (see consideration criteria (j)). 6. VCH is non mutagenic *in vivo* and *in vitro*. Rational for classification of VCH in Carc. Cat. 2 <u>*Cat. 2: Suspected human carcinogens*</u> *The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.* As described above VCH does not fulfil the criteria to classify for Carc. Cat. 1. The only available valid information on carcinogenicity of VCH is derived from an animal study with mice, in which predominantly benign tumor were observed. Tumors in mice may be regarded as a secondary effect of ovarian toxicity. From the toxicokinetic data of VCH sufficient information were provided concerning the differences between mice, rats and humans. The results of these studies suggest that rats are the more appropriate animal model for extrapolation of animal data to humans. Overall evaluation suggest that mechanism causing ovarian tumor is rather mice specific and not relevant to rats or humans. On the other hand reliability of the rat cancer study is very limited. The metabolite VCD, that cause ovarian toxicity and tumors in mice is in principle formed in rats and humans in low amounts as well (in vitro data only). The formation of VCD was proven in vivo in mice but not in rats. Despite amounts formed are considered not to be enough to induce cancer in the rat and probably humans and VCH is non mutagenic in vivo and in vitro, classification of VCH for Carc. Cat. 2 is suggested based on precautionary principle. Conclusion VCH is converted to VCD in a two step process. VCD is supposed to be the active metabolite causing ovarian toxicity in rat and mice and ovarian carcinogenicity in mice. No ovarian	VCD, a classification as Carc. Cat 1B could be more appropriate. Its genotoxic potential deserves to be further investigated *in vivo*. Specific suggestions: Tables 2 and 3 are interesting. Historical incidences have been included in Table 14 (Summary table of relevant carcinogenicity studies) in the CLH report. Section 4.10.4 has been revised in the CLH report to take into account your suggestions. P 22: corrections have been made to clarify the text (Fontaine et al 2001) p37 4.9.6 corrections have	

<table>
<tr><th>Date</th><th>Country / Organisation / MSCA</th><th>Comment</th><th>Dossier submitter's response to comment</th><th>RAC's response to comment</th></tr>
<tr><td></td><td></td><td>
carcinogenicity was found in rats.
The epoxidation of VCH to VCH-1,2-epoxide is the velocity determining step and therefore the critical reaction for ovarian toxicity and carcinogenicity. The epoxidation of VCH to VCH-1,2-epoxide shows distinct differences between mice, rats and humans (Vmax mice >> rat ≥ human).

Detailed toxicokinetic data suggest that mechanism causing ovarian tumor is rather mice specific and not relevant to rats or humans. Existing studies suggest that rats are the more appropriate animal model for extrapolation of animal data to humans (Hoyer&Sipes 2007).

Despite amounts formed are considered not to be enough to induce cancer in the rat and probably humans and VCH is non mutagenic in vivo and in vitro, classification of VCH for Carc. Cat. 2 according to regulation 1272/2008 (EU GHS) is suggested at worst based on precautionary principle as there are still uncertainties e.g. low reliability of NTP rat results.

Carc. Cat. 2 according to regulation 1272/2008 (EU GHS) is the same classification as harmonized EU classification in force for the metabolite VCD. VCD seems to be the responsible metabolite for carcinogenic effects as also discussed in the French CLH dossier. Classifying VCH more restrictive than the metabolite VCD, which is supposed to be the active toxicant, is inappropriate.

Direct comments:
<table>
<tr><th></th><th>Incorrect or miss leading description</th><th>Correction</th></tr>
<tr><td>Page 17 - 24</td><td>4.1 Toxicokinetics (absorption metabolism distribution and elimination)</td><td>A more deliberative description is necessary and should include statements and table 1 of metabolism discussion given above</td></tr>
<tr><td>Page 22</td><td><s>Additionally, Fontaine and coworkers have demonstrated that human CYP "Supersomes" (human CYP + P450 reductase + cytochrome b5) are able to catalyze VCH epoxidation, resulting in the formation of mono- and diepoxide metabolites of VCH (Fontaine et al., 2001).</s></td><td>It has been shown that "Supersomes" containing purified human CYP + purified human P450 reductase + cytochrome b5 and other cofactors in excess that VCH mono epoxide is formed from VCH and in another experiment formation of VCH di epoxide from VCH mono epoxide was shown in this really artificial system (Fontaine et al 2001b). Formation of VCD from VCH</td></tr>
</table>
</td><td>been made to clarify the text

p 41 We do not agree that the NTP study may be considered as not assignable to invalid.

P 44: Partially agree. Corrections have been made to clarify the text

P44-45: Partially agree. Corrections have been made to clarify the text

P46: see our remark on the carcinogenic potential of VCD above

Please see CLH report for references.</td><td></td></tr>
</table>

Date	Country / Organisation / MSCA	Comment			Dossier submitter's response to comment	RAC's response to comment
				therefore is theoretically possible (purified enzymes and high amounts of VCH mono epoxide are needed). However, the group was not able to show direct formation of VCH di epoxide from VCH in this test system (Fontaine et al 2001a).		
		Page 37	**4.9.6 Conclusions on classification and labelling** ~~Information regarding mutagenicity are displayed as supporting evidence for the carcinogenicity endpoint due to the positive *in vitro* results of VCD. However, no classification is discussed and proposed for this endpoint for VCH.~~	4.9.6 Conclusions on classification and labelling Information regarding mutagenicity are displayed as supporting evidence for the carcinogenicity Endpoint. Positive *in vitro* results of VCD are given as additional information as VCD is discussed as possible ultimate carcinogen. However, no classification is discussed and proposed for this endpoint for *VCH*. VCH is clearly non-mutagenic in *in vitro* and *in vivo* OECD Guideline studies. VCH did not produce increase in revertants in TA1537 with or without metabolic activation (rat or hamster S9). In contrast to VCD was positive in the Ames test for TA1537 with metabolic activation. And equivocal results were obtained for TA 1537 without metabolic activation (NTP, 1989). It is known that S9 mix is containing microsomes and enriched with necessary enzymes for VCD generation (eg. CYP2A and CYP 2B).		
		Page 41	4.10.1 Carcinogenicity	A more deliberative description is necessary and should include: "The high dose results are questionable		

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				for rat and mice due to high mortality. Additionally, low dose results are questionable for male rat as well. Reliability of the study may be considered as not assignable to invalid as only results of low dose male and female mice and low dose female rats can be discussed." Consequently results of the NTP study are questionable and can provide only limited evidence therefore.		
		Page 43-45	4.10.4 summary and discussion of carcinogenicity	4.10.4 "summary and discussion of carcinogenicity" is described misleading and therefore wrong assumption are made. A more deliberative description is necessary and have to include statements and tables (2 and 3) of carcinogenicity discussion given above		
		Page 44	~~Since it was demonstrated that human hepatic microsomes and human CYPs are able to catalyse *in vitro* the epoxidation of VCH in mono- and diepoxide (VCD), it cannot be ruled out that this reaction could occur in women exposed to VCH. Although the study available regarding human metabolism of VCH seems to show that human is less potent to transform it into its monoepoxide, VCH-1,2-epoxide, than rat (and subsequently than mouse), some metabolism still occurs. Nevertheless, information about the levels of VCD formed *in vitro* in human hepatocytes is missing.~~	Since it was demonstrated that human hepatic microsomes are able to catalyse in vitro the epoxidation of VCH in mono epoxide and isolated purified human CYP is able to form the diepoxide (VCD) from VCH monoepoxide, it is theoretically possible that VCD is formed in man and women exposed to VCH. Although the study available regarding human metabolism of VCH seems to show that human is less potent to transform VCH into its monoepoxide (VCH-1,2-epoxide) than rat (and subsequently than mouse), some metabolism still occurs. Information about the levels of VCD formed in vitro in human hepatocytes is missing. However, studies with human CYP "Supersomes" (isolated human		

Date	Country / Organisation / MSCA	Comment			Dossier submitter's response to comment	RAC's response to comment
				CYP + P450 reductase + cytochrome b5) failed to demonstrate direct VCD formation from VCH (Fontaine et al. 2001a).		
		Page 44 - 45	Overall, the only valid study to assess carcinogenic effects of VCH is the oral carcinogenicity study in female mice exposed to VCH. The results observed with male mice and female and male rats could not be used to evaluate the hazard potential of VCH because of the poor survival in these animals. However, based on the welldescribed mechanism by which ovarian tumors are produced (metabolisation of VCH in VCD and subsequent destruction of small oocytes), and on the fact that *in vitro* epoxidation of VCH was observed in human hepatocytes, it cannot be ruled out that this mechanism is relevant to human.	VCH carcinogenicity study of NTP in rats and mice suffer from high mortality due to VCH toxicity. Overall, the only valid study parts to assess carcinogenic effects of VCH are the low dose oral carcinogenicity in mice and in female rats exposed to VCH. The results observed with male high dose mice and male rats and high dose female rats can not be used to evaluate the hazard potential of VCH because of the poor survival in these animals. Based on the well-described mechanism by which ovarian tumors are produced (metabolisation of VCH in VCD and subsequent destruction of small oocytes) it can be concluded that VCH can lead to ovarian tumors in mice. However, relevance of this result for rat and humans cannot be concluded from the available data. The epoxidation of VCH shows distinct differences between mice, rats and humans. From the existing data it can be concluded that formation of relevant amounts of the toxic metabolite VCD is rather mouse specific. In vitro studies have proven VCD formation directly from VCH in mice but theses studies fail to show VCD formation directly from VHC for rat and human supersomes (Fontaine et al. 2001a). Existing toxicokinetic studies suggest that rats are the appropriate animal model for extrapolation of animal data to humans (Hoyer&Sipes 2007).		

Date	Country / Organisation / MSCA	Comment			Dossier submitter's response to comment	RAC's response to comment
				However, some uncertainty remains, despite formation of relevant amounts of VCD is unlikely. Theoretically VCD formation in an amount below the detection limit is possible (Fontaine et al. 2001b).		
		Page 45-46	4.10.5 Comparison with criteria	Comparison with criteria for classification pp45 - 46 has to be changed. Based on the comments given above the assumption made in chapter 4.10.5 are not correct or incomplete, respectively. See discussion given above.		
		Page 46	4.10.5 Comparison with criteria	Carc. Cat. 2 according to regulation 1272/2008 (EU GHS) is the classification as harmonized EU classification in force for the metabolite VCD. VCD seems to be the responsible metabolite for carcinogenic effects also discussed in the French CLH dossier. Classifying VCH more restrictive than the metabolite VCD, which is supposed to be the active toxicant, is inappropriate.		

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Flaws JA, Doerr JK, Sipes IG, Hoyer PB (1994): Destruction of preantral follicles in adult rats by 4-vinyl-1-cyclohexene diepoxide. Reprod. Toxicol. 8: 509-514.

Fontaine SM, Mash EA, Hoyer PB, Sipes IG. (2001a). Stereochemical aspects of vinylcyclohexene bioactivation in rodent hepatic microsomes and purified human cytochrome p450 enzyme systems. Drug Metab Dispos. 29 (2): 179-18.

Fontaine SM, Hoyer PB, Halpert JR, Sipes IG (2001b): Role of induction of specific hepatic cytochrome P450 isoforms in epoxidation of 4-vinylcyclohexene. Drug. Metab. Dispos. 29 (9): 1236-1242.

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Hoyer PB, Sipes IG (2007): "Development of an Animal Model for Ovotoxicity Using 4-Vinylcyclohexene: A Case Study". Birth Defects Research (Part B) 80:113–125. Keller DA, Carpenter SC, Cagen SZ, Reitman FA (1997): In vitro metabolism of 4-vinylcyclohexene in rat and mouse liver, lung, and ovary. Toxicol. Appl. Pharmacol. 144 (1): 36-44. NTP Technical Report on the Toxicology and carcinogenesis studies of 4-vinylcyclohexene (CAS N°. 100-40-3) in F344/N rats and B6C3F1 mice. US National Toxicology Program, NTP TR 303, August 1986. NTP Technical Report on the Toxicology and carcinogenesis studies of 4-vinyl-1-cyclohexene diepoxide (CAS 106-87-6) in F344/N rats and B6C3F1 mice (dermal studies). US National Toxicology Program, NTP TR 362, 1989. Rajapaksa KS, Cannady EA, Sipes IG, Hoyer PB (2007): Involvement of CYP 2E1 enzyme in ovotoxicity caused by 4-vinylcyclohexene and its metabolites. Toxicol. Appl. Pharmacol. 221 (2): 215-221. Smith BJ, Carter DE, Sipes IG (1990a): Comparison of the disposition and in vitro metabolism of 4-vinylcyclohexene in the female mouse and rat. Toxicol. Appl. Pharmacol. 105 (3): 364-371. Smith BJ, Mattison DR, and Sipes IG (1990b): The role of epoxidation in 4-vinylcyclohexeneinduced ovarian toxicity. Toxicol. Appl. Pharmacol. 105 (3): 372-381. Smith BJ, Sipes IG (1991): Epoxidation of 4-vinylcyclohexene by human hepatic microsomes. Toxicol. Appl. Pharmacol. 109 (2): 367-371.		
13/07/2011	Sweden / Member State	The Swedish Chemicals Agency (KemI) agrees with the submitting MS that the data available are sufficient for classification of 4-vinylcyclohexene (VCH) as Carc. Cat. 1B according to Reg. 1272/2008 and as Carc. Cat. 2 according to Dir. 67/548/EEC. Page 52. As the MS has not received any further information it could be interesting to compare the suggested classification to "Notifications for classification and labeling" submitted to ECHA for VCH (REACH-IT).	Thanks for your support. Notifications integrated pg 52	Noted
13/07/2011	Belgium Cefic / BehalfOfAnOrganisation / Industry or trade association	I am writing behalf of the Lower Olefins Sector Groups of Cefic, the Acrylonitrile Butadiene Styrene Copolymer - Styrene Acrylonitrile Copolymer group of Plastics Europe and the International Institute of Synthetic Rubber Producers. *ECHA comment: The document attached "Letter from Cefic, PlasticsEurope and SRP,12/07/2011, 4-vinyl*	Please see our response to comment above from Germany / AffiliatedWithOrga	Noted

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		cyclohexene (VCH) Response to proposal for harmonised classification and labelling" (CEFIC Letter re 4VCH.pdf)) is copied below: **4-vinyl cyclohexene (VCH)** **Response to proposal for harmonised classification and labelling** On behalf of the Lower Olefins Sector Groups of Cefic, the Acrylonitrile Butadiene Styrene Copolymer - Styrene Acrylonitrile Copolymer group of Plastics Europe and the International Institute of Synthetic Rubber Producers, we are writing to provide comment on the proposal from France for a harmonised classification and labelling for 4-vinyl cyclohexene (VCH). Whilst agreeing with the general interpretation of the criteria document we believe that some critical issues are only partially covered, and we specifically disagree with the interpretation of the findings against the criteria for classification for the cancer end point. The basis for our divergence of opinion are set out in the accompanying document. We trust that you will agree with our concerns and look forward to receiving your considered opinion. Yours faithfully, Graeme Wallace, Manager, Aromatics & Olefins – Cefic Philippe Marechal, Manager, Styrenics Chain – Plastics Europe James L. McGraw, Managing Director & CEO, International Institute of Synthetic Rubber Producers The document mentioned in this letter is copied in the comment on carcinogenicity made by Belgium / Graeme Wallace / Cefic / on 13/7/2011 below.	nisation / Company-Manufacturer	

Date	Country/ Person/ Organisation/ MSCA	Comment	Dossier submiter's response to comment	RAC's response to comment
14/07/2011	United Kingdom / Member State	We agree that the mouse ovarian tumours support classification for carcinogenicity; however, we are not sure the available information justifies the Category 1B proposal. We are not convinced that hepatic generation of reactive epoxides and transport to the ovary is a plausible explanation for the ovarian tumours. Given the likely reactivity of the VCH epoxide (and diepoxide) and the widespread systemic distribution the proposed mode of action requires, it is surprising that no tumours were induced in the liver and non ovarian tissues. There was no evidence of genotoxicity from the limited information available. In addition, a non-genotoxic mode of action is supported by the lack of tumour findings in other tissues in the mouse, and the negative rat carcinogenicity study. In conclusion, taking account of the apparent lack of genotoxicity and uncertainties surrounding the proposed mode of action, we consider that CLP Category 2 is more appropriate.	According to a comprehensive review on the VCH-mediated ovotoxicity by Hoyer and Sipes, 2001, VCH or VCD "cause small preantral follicle loss by a direct targeting of the ovary". Then, VCH or its epoxide metabolites are expected to reach the ovary. Maybe the liver metabolism is not the only one to contribute to the metabolism of VCH. Indeed, although no epoxidation of VCH or its monoepoxides was detected in mouse or rat ovary incubated with VCH in vitro (Keller et al., 1997), Cannady et al. (2003)	We thank the MSCA for including more information on the MoA, especially about the targeting of the ovary. We agree with the MSCA that it is not demonstrated that the MoA is not relevant for humans, and we agree that no firm conclusion can be drawn with regard to the genotoxic potential of VCH. However, based on available data of mutagenicity of VCH, concern is low. After a weight-of evidence analysis we regard category Carc. 2 (CLP) as the most appropriate for VCH. Hence we do not support the dossier submitters

			demonstrated that VCH/VCD are able to induce CYP2E1, CYP2A and CYP2B in F1 follicles (but also in F3 follicles and interstitial cells) from mice previously exposed to VCH or VCD for 15 days. This would demonstrate that an induction of CYPs in the ovary may activate the metabolism of VCH or VCD in the ovary, the extent of this reaction in ovary compared to that in liver being unknown. In addition, we are of the opinion that no firm conclusion can be drawn about the genotoxic potential of VCH and VCD and that this endpoint has not been sufficiently investigated. Indeed, as mentioned in the CLH dossier (4.9.4 Summary and discussion of mutagenicity), in	proposalt the cat 1B proposal in regard to carcinogenicity. For more reasoning, please see the RAC opinion.

			vitro systems may be inappropriate to test VCH since rat S9 may fail to metabolise VCH into the ultimate metabolite VCD. Interestingly, a mouse lymphoma assay was found positive in a NTP study (not published but results available on the NTP website (http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=mouselymphoma.studyDetails&study_no=971117&cas_no=100-40-3&endpointlist=ML,ML-N)). In vivo genotoxicity was investigated by a micronucleus assay in rats and mice exposed to VCH by inhalation for 2 days or 13 weeks (Bevan *et al.*, 2001). However, validity of the results is questioned since only 1000 PCE per animal were scored (the actual OECD 474 TG recommends to	

			score a minimum of 2000 immature erythrocytes per animal for the incidence of micronucleated immature erythrocytes), no individual data are available, and no historical negative/positive control data are available (especially for 1,3-butadiene used as a positive control in the mouse micronucleus assay)). With regard to VCD, no in vivo assay is available, although it is mutagenic in several in vitro tests and carcinogenic in rats and mice. However, it was found to form DNA adducts (Randerath and Mabon, 1996). Finally, it should be emphasised that increased incidence of tumors were observed in rats but, due to poor survival, the results may be	

			misleading. Early excessive mortality may have masked the higher outcome of tumors induced by VCH. Nevertheless, the increased incidence of adenomas or squamous-cell carcinomas (combined) of the clitoral gland in low-dose female rats is considered since the survival of these animals is similar to control until week 102. Overall, although a MOA by which VCH-induced ovary carcinogenesis could occur via oocyte depletion leading to ovary tumors is plausible, there is no evidence that a genotoxic component does not play a role in the VCH carcinogenicity. Please see CLH report for	

			references	
30/06/2011	Germany / Member State	The carcinogenicity studies (NTP TR 303) in rats and mice performed with VCH suffer from premature mortalility, which indicates excessive dosing. For classification purposes it is important to get more information this aspect, e. g. whether MTD is exceeded (see Lit 1). So, precise values of body weight gain in the different groups are needed. The NTP-report (NTP TR 303, table 8) states that e. g. female mice of the low dose group (200 mg/kg) started with an average body weight, which was 113% of the control animals, but ended with an average body weight, which was 99% of the control. Similar values were found for the high dose females (400 mg/kg; 114% in the beginning of dosing and 99% at the end). The reduced body weight gain of animals, considering the initial problem to randomize animals (NTP TR 303, p32-43), should be presented in more detail. Concerning the rat study it is mentioned in the text of table 14 of the CLP-report that the low dose and the high females showed a statistically significant increase of mortality. The respective asterisk is missing at the low dose figure (22/50). Please clarify. Different kind of tumours seemed to occur in the ovary. It should be clearly stated whether these are considered as malignant or benign. It is described in the CLH-report, that VCD, classified with Carc. Cat. 2, is the ultimate metabolite and toxicant. Furthermore it is stated that metabolism from VCH to VCD is different in species, showing the highest activity in mice, followed by rats. The lowest activity is assorted to humans. This should also be considered, if a study in mice is considered as the only carcinogenicity study performed with VCH. Overall, there are some uncertainties about a clear-cut classification as Carc. Cat. 1b. Both studies suffer from excessive dosing and the CLH-report states, that the study in female mice is the only reliable study performed with VCH. Only one dose in female mice did not result in premature mortality. These aspects should be discussed in more detail in chapter 4.10.5 of the CLH-report with respect to the Guidance. Lit: 1: Guidance on the Application of the CLP Criteria: Guidance to Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of substances and mixtures, chapter 3.6.2.3.2. (j) The possibility of a confounding effect of excessive toxicity at test doses	We do not think that ovary tumors observed in mice could be confounded with “excessive toxicity at test doses” since the incidence is statistically significant even at the low dose at which mortality was similar to control. Table 8 summarized survival and body weigth data for rats. According to Table 15, randomization of mice at the beginning of the study is acceptable (98% for low-dose female mice and 100% for high-dose female mice). At week 100, body weight is reduced to 93 and 88% of the control value, in low- and high-dose female mice, respectively. Therefore, no excessive toxicity	We agree with the MSCA that the ovary tumors observed in mice could not be confounded with “excessive toxicity at test doses”. We note that VCD is classified in Carc Cat 2 in the CLP regulation today. We thank the MSCA for providing additional information for comparison with the criteria to classify VCH.

			is observed in low-dose female mice. Criteria to classify VCH in Carc Cat 1B have been revised in the CLH dossier Concerning the mortality of female rats, the incidence is increased (22/50 vs 10/50 in controls) but not significantly. The increase is significant only after week 102 (P value = 0.022)	
01/07/2011	Ireland / Health and Safety Authority / BehalfOfAnOrganisation	The Irish CA is in agreement with the proposed classification of Carc. 1B- H350 (Carc. Cat. 2; R45).	Thanks for your support	Noted. However we find that based on the available data classification in category Carc.2 (CLP) is more apropriate for VCH. For arguments of this, please see the opinion and elsewhere in this RCOM.
05/07/2011	Netherlands / Bureau REACH / Member State	P38, table 14: Despite the high mortality in rats, several treatment-related tumours were observed in various parts of the body, including squamous-cell papillomas or carcinomas of the skin (males) and adenomas or squamous-cell carcinomas (combined) of the clitoral gland (females). Incidences are increased at the end of the study (only survivers), but even when all animals are included in the analysis. Therefore, these data may provide additional signs that vinylcyclohexene has a carcinogenic potential and should not simply be ignored. P38, table 14: Despite the high mortality male mice, treatment-related tumours were observed in various parts of the body, including malignant lymphomas and alveolar/bronchiolar adenomas or carcinomas (combined) of the	Thanks for your support. We acknowledge that increased tumor incidence occurred in male mice and male and female rats.	We agree with the MSCA and thank them for including this information.

		lung. Therefore, besides the data on ovarian neoplastic lesions in female mice, these data in male mice may provide additional signs that vinylcyclohexene has a carcinogenic potential and should not simply be ignored. P41: 4.10.1.1: see comments above.	However, early excessive mortality may have masked increased incidences of different types of tumors. Nevertheless, the increased incidence of adenomas or squamous-cell carcinomas (combined) of the clitoral gland in low-dose female rats is considered since the survival of these animals is similar to control until week 102. The CLH report has been revised to take into account your comments.	
06/07/2011	United States / Individual	Pages 38-45. Section 4.10. A MOA has been proposed for the ovarian tumors in mice from VCH exposure (Bevan et al., 2009). The MOA was evaluated using the modified Hill criteria for causality in the IPCS Human Relevance Framework. A manuscript is in preparation. Proposed Mode of Action for Mouse Ovarian Tumors Following exposure and uptake, VCH is metabolized, primarily in the liver, to VCH-1,2-epoxide or VCH-7,8-epoxide, which are further metabolized to VCH-diepoxide. VCH-diepoxide enters the blood and circulates through the body. Upon reaching the ovary, VCH-diepoxide selectively destroys the primordial and primary follicles through a mechanism involving programmed cell death or apoptosis. Repeated exposures to VCH ultimately result in premature ovarian failure, due to complete follicular loss. Since 17β-estradiol and inhibin are no longer produced from the primordial and primary follicles in the ovary, loss of the negative feedback inhibition of FSH release from the hypothalamus and pituitary occurs, leading to high plasma levels of FSH. Increased plasma levels of FSH results in the initiation and/or promotion of ovarian tumors.	Thank you for these interesting data on the MOA of VCH-induced ovary tumors. The potential of VCH to be a endocrine disruptor is noted. The CLH report has been revised to provide more information on this MOA, based on your communication and on the review	We are grateful to Dr Bevan for submitting valuable information about the MoA and to the MSCA for including it in the revised report. We note an editorial error in the key events, and assume that the word “decreased”

		Key Events Following exposure and uptake of VCH, the key events leading to ovarian toxicity and tumors outlined below (a table with references is also provided as an attachment). 1. Systemic levels of VCHD 1a. Bioactivation of VCH to VCHD (via VCH-1,2-epoxide) 1b. Hydrolysis of VCH epoxide metabolites by epoxide hydrolase 2. Decreased follicular loss in ovaries from VCHD 3. Selective destruction of primordial and primary follicles through apoptosis 4. Ovarian failure (no estrous cyclicity) from complete oocyte loss 5. Increased plasma FSH levels from release of negative feedback of 17β-estradiol and inhibin on hypothalamus and pituitary. 6. Initiation and/or promotion of ovarian tumors from increased plasma FSH levels. Chronic oral exposure of female mice to VCH resulted in ovarian granulosa cell tumors (Collins et al., 1987). Preceding the tumors, a reduction in the number of follicles, particularly the primary follicles, were noted in the ovaries of female mice exposed orally or by inhalation for 13 weeks to VCH (Collins and Manus, 1987; Bevan et al., 1996). Rats exposed to VCH did not show any ovarian toxicity or increased incidence of ovarian tumors, although it is difficult to reach any strong conclusions about the ovarian tumor incidence in rats because of poor survival in the oral chronic study (Collins and Manus, 1987; Collins et al., 1987; Bevan et al., 1996). VCH-diepoxide (VCHD) is the metabolite responsible for the ovary toxicity (follicular destruction). Analogues of VCH that have the potential to form only a monoepoxide metabolite failed to deplete small follicles (Hooser et al., 1993; Doerr et al., 1995), whereas compounds that form diepoxides, such as 1,3-butadiene and isoprene, significantly depleted follicles (Doerr et al., 1995). The species difference in ovarian toxicity appears largely due to differences in the rate of bioactivation of VCH to VCH epoxides (VCH-1,2-epoxide, VCH-7,8-epoxide and VCH-diepoxide) by cytochrome P-450 enzymes. The balance of activation versus detoxification reactions of VCH metabolism are different between mice and rats. In general, the mouse is more efficient at metabolism of VCH to epoxides than is the rat. In contrast, the rat may be more efficient at hydrolysis of epoxides. Thus, the rat would tend to have a lower body burden of epoxide metabolites than the mouse at equal doses of VCH, which would explain why the rat is not suspectible to ovarian toxicity from VCH. If, however, the ultimate metabolite VCHD is administered to either rats or mice, metabolism does not play a limiting role in the ability of the diepoxide to be formed in sufficient quantity so that it can reach the ovary and target primordial and primary follicles. No VCH metabolism data exist for nonhuman primates. Data on olefinic compounds, such as 1,3-butadiene, indicate that nonhuman primates are similar to humans with respect to cytochrome P-450 bioactivation of olefins to its epoxide metabolites (Dahl and Henderson, 2000). Limited in vitro data with human liver microsomes suggest that VCH metabolism in nonhuman primates is likely to be more like the rat than the mouse (Smith and Sipes, 1991). The follicles that are selectively targeted by VCHD are the primordial and primary follicles (Springer et al., 1996).	from Hoyer and Sipes (2007).	should be replced with “increased”: “2. Decreased follicular loss in ovaries from VCHD”

		The pathological changes in the ovary when mice are dosed with either VCH or VCHD or when rats are dosed with VCHD are identical (Flaws et al., 1994; Springer et al., 1996; Mayer et al., 2002). VCHD selectively destroys the primordial and primary follicles through a mechanism involving programmed cell death or apoptosis, thereby accelerating the normal process of atresia (Springer et al., 1996; Hoyer and Sipes, 2007). Ovarian failure (premature menopause) is a consequence of VCH-induced primordial and primary follicle loss (Hooser et al., 1994; Mayer et al., 2002). In mice given daily intraperitoneal injections of 800 mg/kg VCH for 30 days, there was >90% loss of the small pre-antral follicles at the end of the dosing period (Hooser et al., 1994). At 240 days of the study (210 days following VCH treatment), there were few widely scattered oocytes in small and growing follicles; however, at 360 days, no oocytes at any stage were observed in the VCH-treated mice. The complete loss of oocytes at 360 days coincided with the loss of estrous cyclicity, indicating ovarian failure. Follicular loss also resulted in increased follicle stimulating hormone (FSH) plasma levels, presumably due to the lack of 17β-estradiol and inhibin production from the follicles. 17β-Estradiol and inhibin exert negative feedback inhibition of FSH production in the hypothalamus and/or pituitary. Plasma FSH levels were not elevated above control levels until 240 days following the initiation of dosing, suggesting that virtually complete loss of follicles is needed before the release of the negative feedback inhibition at the hypothalamus/pituitary. At the time of ovarian failure, VCH-treated mice showed lesions in the ovary that appear similar to preneoplastic lesions reported in a genetically susceptible strain of mice for granulosa cell tumors (Hooser et al., 1994; Tennant et al., 1990). A similar pattern was reported for rats dosed intraperitoneally for 30 days with 80 mg/kg VCH-diepoxide (Mayer et al., 2002). Rats dosed with VCH-diepoxide had reduced number of preantral follicles by day 30. Following cessation of dosing, relative to controls, primordial, primary, and secondary follicles were progressively lost with time. Circulating FSH levels in VCH-treated rats were greater (days 120, 240 and 360) than in controls. Cyclicity was disrupted in the VCH-diepoxide treated animals by day 360. VCHD has been shown to selectively deplete primordial and primary follicles in the ovaries of nonhuman primates (Macaca fascicularis) (Appt et al., 2006). Several animal models initially drew attention to the possible involvement of gonadotropins in ovarian tumorigenesis. Biskind and Biskind (1944) reported a high incidence of ovarian tumors in rats whose ovaries were autotransplanted to the spleen. However, the formation of the ovarian tumors did not occur when one ovary was left intact or when the ovary was autotransplanted in previously hypophysectomized animals. (Biskind and Biskind, 1948). This tumorigenesis has been attributed to elevated pituitary gonadotropins due to the deactivation of estrogen in the liver and the consequent depletion of negative feedback of estrogen on the pituitary. Since then, the development of ovarian tumors has been reported in several transgenic or knockout animal models that exhibit hypergonadotropism with high levels of circulating FSH and LH similar to the postmenopausal state in women (Kumar et al., 1999; Risma et al., 1995). Granulosa cell tumors can also be induced by genetic deletion of germ cells (Murphy, 1972; Murphy and Beamer, 1973), neonatal thymectomy (Nishizuka et al., 1972), or X-irradiaton (Marchant, 1987). The hormonal tumorigenesis hypothesis for ovarian granulosa cell cancers is that endocrine factors that control the normal growth of target organs can also provide suitable conditions for neoplastic transformation. The gonadotropin hypothesis has been proposed as an underlying mechanism to ovarian cancer, in that excessive levels of gonadotropins, related to the surge occurring during ovulation and the loss of gonadal negative feedback in		

		menopause and premature ovarian failure (oocyte depletion), may play a role in the development and progression of ovarian (granulosa cell) cancer perhaps through alteration in signaling pathways affecting cell growth (Murphy, 1980; Fuller et al., 2002). The incidence of ovarian cancer in women climbs dramatically around the age at which most women reach menopause. The onset of menopause, which happens at approximately 51 years of age, involves changes in gonadotropin levels as a result of cessation of ovarian function and menstrual cycle. The complete cessation of ovarian function results in the loss of negative feedback of ovarian steroids (i.e., 17β-estradiol) on gonadotropins. In 2 to 3 years after menopause, gonadotropin levels are particularly high, such that the concentrations of FSH and LH reach a peak of 10-20 times and 3-4 times the values recorded during the proliferative phase of the menstrual cycle, respectively (Chakravarti et al., 1976; Speroff et al., 1999). The increase in plasma gonadotropin levels is a result of the loss of feedback inhibition from 17β-estradiol and inhibin, both of which are produced from follicles. In the case of ovarian failure where there is complete loss of oocytes in the ovary, the loss of 17β-estradiol and inhibin from the follicles leads to increased plasma gonadotropin levels. References Appt, S.E., Kaplan, J.R., Clarkson, T.B., Cline, J.M., Christian, P.J., and Hoyer, P.B (2006) Destruction of primordial ovarian follicles in adult cynomolgus macaques after exposure to 4-vinylcyclohexene diepoxide: a nonhuman primate model of the menopausal transition. Fert. Steril. 86(Suppl. 3): 1210-1216. Bevan, C., Stadler, J.C., Elliott, G.S., Frame, S.R., Baldwin, J.K., Leung, H.-W., Moran, E., and Panepinto, A.S. (1996) Subchronic toxicity of 4-vinylcyclohexene in rats and mice by inhalation exposure. Fundam. Appl. Toxicol. 32: 1-10. Bevan, C., Gargas, M., Kirman, C., and Vergnes, J. (2009) Mode of action (MOA) evaluation and derivation of a cancer and non-cancer reference value for 4-vinylcyclohexene. Toxicol. Sci. 108 (Suppl.), abstract #839 Biskind, M.S., and Biskind, G.R. (1944) Development of tumors in the rat ovary after transplantation into the spleen. Proc. Soc. Exp. Biol. Med. 55: 176-179. Biskind, M.S., and Biskind, G.R. (1948) Atrophy of ovaries transplanted to the spleen in unilaterally castrated rats; proliferative changes following subsequent removal of intact ovary. Science 108: 137-138. Chakravarti, S., Collins, W.P., Forecast, J.D., Newton, J.R., Oram, D.H., and Studd, J.W. (1976) Hormonal profiles after the menopause. Br. Med. J. 2: 784-787. Collins, J.J., and Manus, A.G. (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. Health 21: 493-505. Collins, J.J., Montali, R.J., and Manus, A.G. (1987) Toxicological evaluation of 4-vinylcyclohexene: II. Induction of ovarian tumors in female B6C3F1 mice by chronic oral administration of 4-vinylcyclohexnene. J. Toxicol.		

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12/07/2011	Germany / BehalfOfAnOrganisation / Company-Manufacturer	Please find our comments concerning evaluation of carcinogenicity in the enclosed attachment. *ECHA comment: View document attached: Comments from Evonik Industries, 12/07/2011, (evonik_statement_CLH_VCH_France.pdf).*	Please see our response to comment above from Germany / AffiliatedWithOrganisation / Company-Manufacturer	Noted
13/07/2011	Belgium / Cefic / BehalfOfAnOrganisation / Industry or trade association	We disagree with the interpretation of the findings against the criteria for classification for the cancer end point. Our comments are in the zip file *ECHA comment: The document attached "4-vinyl cyclohexene (VCH) Response to proposal for harmonised classification and labelling" (VCH-Comments.docx) is copied below:* Introduction The Lower Olefins Sector Groups of Cefic, the Acrylonitrile Butadiene Styrene Copolymer - Styrene Acrylonitrile Copolymer group of Plastics Europe and the International Institute of Synthetic Rubber Producers welcomes the opportunity to comment on the proposal from France for a harmonised classification and labelling for 4-vinyl cyclohexene (VCH). The criteria document (dated May 2011) fairly reviews the existing data and we agree with the general interpretation. We do, however, believe that some critical issues are only partially covered and we specifically disagree with the interpretation of the findings against the criteria for classification for the cancer end point. We believe an excessively conservative interpretation has been made and the reasons for our view are given below.	Please see our response to comment above from : • Germany / AffiliatedWithOrganisation / Company-Manufacturer • United Kingdom / Member State The CLH report	Noted.

		Summary and assessment of the relevant data There is only one cancer study that is reliable and that is the one carried out with female mice. This study showed a clear, statistically and biologically significant increase in tumours of the ovaries only. The studies in male mice and both sexes of rat were not deemed reliable due to excessive mortality in the VCH treatment groups compared to controls. (This is the conclusion of the authors of the study and is repeated by the authors of the C&L proposal.). It should also be noted that the high dose level mice also showed high mortality rates leaving only the single low dose animal group from which conclusions could be drawn. Subchronic studies with female mice have demonstrated lesions that can be considered precursors to the neoplastic lesions seen in the cancer study. Similar studies in female rats have not demonstrated such precursor treatment-related changes. The available data suggests that the ovarian effects seen in female mice are not directly caused by VCH itself but rather by epoxide metabolites, and in particular the diepoxide metabolite (VCD). VCD itself causes ovotoxicity in both rats and mice. Toxicokinetic data from both mouse and rat indicate significant species differences. Indeed, mice produce the epoxide metabolites (mono and diepoxide) at a higher rate of formation and mouse epoxide hydrolases detoxify less efficiently compared to the rat. Data generated with human hepatic microsomes suggests that humans metabolise VCH even more slowly than rats. This work by Smith et al (1991) reported that the viability of the human hepatic microsomes used in the study was assessed using a number of techniques and activity levels found were similar to those reported by others in the literature. There does not therefore appear to be any basis for the comment in section 4.1.2. of the proposal document from France effectively questioning the reliability of the results and urging caution in their use because of mooted confounding from prior exposure of the donors to 'drugs or other environmental chemicals'. A significant amount of research on the metabolism, ovotoxicity and species differences in toxicity of VCH has been carried out at the University of Arizona. In fact, nearly half of the published references cited in the French proposal for classification[1] emanates from this group. In a recent comprehensive review of the toxicity of VCH published by these researchers (Hoyer, 2007) they described the mouse as being uniquely susceptible to VCH and concluded that the rat was as a better model than the mouse to predict VCH-induced ovarian toxicity in humans. Whilst the proposal document does contain a lot of detail on metabolism, the mode of action only seems to be partially covered. The ECHA guidance on the application of the CLP criteria to carcinogenicity data (page 308) states: *To establish a mode of action will usually require specific investigative studies over and above the standard carcinogenicity study. All available data must be considered carefully to judge if it can be concluded with confidence that the tumours are being induced through that specific mechanism. The IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for Humans (2007) can be a useful way to construct and present a robust and transparent assessment of such data*. _____ [1] Excluding the references from NTP, IARC and the public databases. In this case, we do not believe that the mode of action (MoA) has been thoroughly considered. A detailed review of	has been revised to provide more information on this MOA, based on the communication of Bevan and on the review from Hoyer and Sipes (2007).	

the MoA for mouse ovarian tumours resulting from exposure to VCH carried out by the Sapphire Group was published as an appendix to the Texas Commission on Environmental Quality development support document for VCH published in January 2011. This review was conducted using the IPCS framework and is reproduced in the appendix to this document. Following IPCS methodology, the proposed MoA is as follows:
Following exposure and uptake, VCH is metabolized, primarily in the liver, to VCH-1,2-epoxide or VCH-7,8-epoxide, which are further metabolized to VCH diepoxide (referred above as VCD). VCH diepoxide enters the systemic circulation. Upon reaching the ovary, VCH diepoxide selectively destroys the primordial and primary follicles through a mechanism involving apoptosis. Repeated exposures to VCH ultimately result in premature ovarian failure, due to complete follicular loss. Since 17β-estradiol and inhibin are no longer produced from the primordial and primary follicles in the ovary, loss of the negative feedback inhibition of FSH release from the hypothalamus and pituitary occurs, leading to high plasma levels of FSH. Increased plasma levels of FSH results in the initiation and/or promotion of ovarian tumours.
The conclusion from the MoA assessment is that VCH acts via a non-genotoxic, threshold mechanism. VCHD (referred above as VCD), the metabolite of VCH, is selectively cytotoxic to oocytes in the ovary resulting in premature menopause, and as a consequence results in increased plasma levels of FSH which acts as a tumour promoter in ovaries.

Comparison with classification criteria

The proposal is for VCH to be classified as a category 1B carcinogen. According to the CLP regulation, a substance classified as category 1B is:

presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from:
- *human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or*
- *animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals."*

Clearly there are no human studies so the evidence can only come from the animal data in this case. The CLP regulation includes criteria for judging sufficient and limited evidence of carcinogenicity. Our opinion of the assessment against these criteria is shown below:

Sufficient evidence of carcinogenicity

Criteria	Finding
A causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign	**NO**. The findings are only seen in mice. Evidence from sub-chronic studies supports this to be a species specific finding.

and malignant neoplasms in two or more species of animals.	
A causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.	**NO**. There is only a single study available.
An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.	**EQUIVOCAL**. The 'both sexes' criteria is not applicable here as the finding is in a female specific organ. The study is reliable but has some shortcomings. The tumour type is unusual but tumours are not found at multiple sites. The evidence suggests that it is a metabolite that is the proximate toxicant and that mice are particularly effective at generating it and poor at eliminating it compared to other species for which data is available, including humans. The most plausible MoA involves a non genotoxic mechanism that would have a no effect level.

Sufficient evidence of carcinogenicity

Criteria	**Finding**
The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, the evidence of carcinogenicity is restricted to a single experiment.	**YES**. This is clearly the case here since the only reliable study is the one in female mice.
The data suggest a carcinogenic effect but are limited for making a definitive evaluation because there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies;	**PROBABLY**: The studies in rats and male mice were rendered unreliable by the high mortality rates. Even in the high dose females, there was a significant increase in mortality. The studies only used two rather than the normal three dose groups. It is likely that the top doses exceed the MTD. This means that there is only a single dose in which the elevated tumour response was seen in the absence of general toxicity.
The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential.	**POSSIBLY**: The report cites a clear increase in granulosa cell tumours or carcinomas (terminal rates 1/39, 9/38, 7/16). However, the rates of granulosa cell carcinomas alone were 0/39, 1/38, 2/16. Bearing in mind the latter result could be confounded by exceeding the MTD, this means a single incidence in the low dose group may be the only unequivocal

	finding of carcinoma.
The data suggest a carcinogenic effect but are limited for making a definitive evaluation because the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.	**NOT RELEVANT**: carcinogenic effects are seen, albeit in a single species.

According to this assessment against the criteria, there is only limited evidence of a carcinogenic effect and therefore a classification category 2 rather than category 1B is most appropriate.
It is important to also consider whether the mutagenicity data supports such a mode of action. From the available data, VCH itself is not mutagenic in an Ames test with or without metabolic activation (the former using standard rat liver S9) nor is it mutagenic using micronucleus assays in rats or mice. The proposal document dismisses the negative findings in the mouse micronucleus study (Bevan et al., 2001) because cyclophosphamide was not used as a positive control. While cyclophosphamide was used as the positive control substance for the assessment of VCH-induced genotoxicity in the rat, the positive control for mice was butadiene (1000ppm). In this study, VCH caused no increase in micronucleated polychromatic erythrocytes (MN-PCE) in the bone marrow of male or female mice at concentrations of up to 1000ppm (2 days or 13 weeks exposure). In contrast, butadiene caused a substantial and statistically significant increase in MN-PCE in both genders over both exposure durations, demonstrating that the test, as conducted, was capable of detecting a positive response and is therefore valid. Overall, the data shows no evidence for mutagenicity which supports the hypothesis that the tumours seen in mice occur via a non-genotoxic mode of action.
The criteria document cites the "Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals" as justification for using the data on VCD to justify a category 1B classification. However, the full paragraph of this section, which is more related to the justification for defining metabolic categories of substances, is as follows:

The underlying hypothesis for a metabolic series is a sequential metabolism of a parent chemical to downstream blood metabolites that are chemicals of interest. Hazard identification studies with the parent compound could then be used to identify the hazards associated with systemic blood levels of the downstream primary and secondary metabolites and once quantified, can be used in place of studies using direct exposure to primary and secondary metabolites themselves. In certain instances, the metabolism of the parent compound within barrier tissue (e.g. lung or gut tissue) occurs so rapidly that the initial primary metabolite is the predominant chemical found within the blood. Under these circumstances data from hazard identification studies conducted with that primary metabolite itself can be used to identify hazards for the parent compound.

This indicates that in order to use a metabolite to support hazard identification of a parent compound, information is needed to quantify resultant systemic blood levels. Such information is not present for VCH, but the data that is available suggests that resultant blood levels will be sufficiently low (taking the rat as a better model than the mouse for the human situation) that no significant hazard exists. This approach of using data on the metabolite as justification for 'sufficient evidence' of carcinogenicity does not appear to be justified according to the full paragraph of guidance in chapter R.6 as there is no available data on quantified systemic blood levels in the test species. This is very important as the proposed mode of action suggests that levels of resultant metabolites are

		critical in determining the hazard from exposure to VCH. The use of the paragraph as cited in the proposal document seems to have been taken out of its intended context. Conclusion In conclusion, we do not believe that there is sufficient evidence to justify classification of VCH as a category 1B carcinogen. The evidence for a causal relationship between oral exposure to VCH and increased incidence of ovarian tumours is actually from a single study in mice that only used two dose levels, the higher of which clearly exceeded the maximum tolerated dose. No other significant tumours were seen in the study. These tumours are not seen in rats. Available data indicate that VCH is not genotoxic in vitro or in vivo. The existing data provide good evidence to support the hypothesis that mouse ovarian tumours occur by a mode of action with a threshold that is not exceeded in rats and is also unlikely to be exceeded in humans because of quantitative differences in metabolism between species. Classification should only be based objectively on the available data rather than on conjecture. The data can only be regarded to support a conclusion of 'limited evidence' of carcinogenicity and therefore only supports a classification of category 2 at worst. References Hoyer PB, Sipes IG (2007). "Development of an Animal Model for Ovotoxicity Using 4-Vinylcyclohexene: A Case Study". Birth Defects Research (Part B) 80:113–125. Smith BJ, Sipes IG (1991) "Epoxidation of 4-vinylcyclohexene by human hepatic microsomes." Tox Appl Pharmac 109(2), 367-71. Appendix. This section is the reproduced appendix B from the TCEQ development support draft document published for comment in January 2011 and is an evaluation of the proposed mode of action for mouse ovarian tumours. TCEQ website address: http://www.tceq.texas.gov 4-Vinylcyclohexene -Proposed Pages 48-62 Appendix B Sections 5.0 and 5.1 from the Sapphire Group (2008) 5.0 Mode of Action(s) of Mouse Ovarian Tumors An evaluation of the mode of action (MOA) by which VCH produces ovarian tumors in rodents was conducted using the IPCS Human Relevance Framework (Meek et al.,2003; Boobis et al., 2006). In this framework, three fundamental questions are considered for theMOA: 1.Is the weight of evidence sufficient to establish an MOA in animals? 2.Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans? 3.Can human relevanceof the MOA be reasonably excluded on the basis of quantitative differences in either kinetic		

		or dynamic factors between experimental animals and humans? Following a consideration of these three questions, a confidence statement is given, along with a discussion of the implications of the MOA to the risk assessment. 5.1. Proposed Mode of Action for Mouse Ovarian Tumors Following exposure and uptake, VCH is metabolized, primarily in the liver, to VCH-1,2-epoxide or VCH-7,8-epoxide, which are further metabolized to VCH-diepoxide. VCH-diepoxide enters the blood and circulates through the body. Upon reaching the ovary,VCH-diepoxide selectively destroys the primordial and primary follicles through amechanism involving programmed cell death or apoptosis. Repeated exposures to VCH ultimately result in premature ovarian failure, due to complete follicular loss. Since 17-estradioland inhibin are no longer produced from the primordial and primary follicles inthe ovary, loss of the negative feedback inhibition of FSH release from the hypothalamusand pituitary occurs, leading to high plasma levels of FSH. Increased plasma levels ofFSH results in the initiation and/or promotion of ovarian tumors. 5.1.1. Key Events Following exposure and uptake of VCH, the key events leading to ovarian toxicity andtumors are presented in Table 4 and outlined below. 1.Systemic levels of VCHD 1a. Bioactivation of VCH to VCHD (via VCH-l ,2-epoxide) 1 b. Hydrolysis of VCHepoxide metabolites by epoxide hydrolase 2.Decreased follicularloss in ovaries from VCHD 3.Selective destruction of primordial and primary follicles through apoptosis 4.Ovarian failure (no estrous cyclicity) from complete oocyte loss 5.Ovarian failure (no estrous cyclicity) from complete oocyte loss 6.Increased plasma FSH levels from release of negative feedback of 17β-estradiol and inhibin on hypothalamus and pituitary. 7.Initiation and/or promotion of ovarian tumors from increased plasma FSH levels.		

Table 4 Key Events in the Proposed MOA for VCH-Induced Ovarian Tumors

Key Events	Evidence in Animals	Confi-dence	Key References
1. Systemic levels of VCH diepoxide	Blood levels inferred from studies showing blood levels of VCH-1,2-epoxide in VCH-dose mice, and from toxicity studies with VCHD	High	Smith et al. 1990 a,b,c; Keller et al. 1997
1a. bioactivation of VCH to VCH diepoxide (via VCH-1,2-epoxide)	*In vitro* liver, lung, and ovary microsome studies, with formation greater in mice than rats. Inhibition of cytochrome P450 reduces VDH-1,2-epoxide formation *in vivo* and *in vitro*	High	
1b hydrolysis of VCH epoxide metabolites by epoxide hydrolase]	*In vitro* liver, lung and ovary microsome studies, with rats having higher epoxide hydrolase rates than mice	High	
3. Selective destruction of primordial and primary follicles through apoptosis	Secondary follicles not directly affected by VCHD treatment; morphological and biochemical pathway studies.	High	Hooser et al. 1994; Flaws et al. 1994; Springer et al. 1996; Kao et al. 1999; Mayer et al. 2002 ; Hu et al. 2001a,b ; 2002
4. Ovarian failure (no estrous cyclicity) from complete oocyte loss	Long-term studies (up to one year) from 30 day treatment with either VCH (mice) or VCHD (rats);	High	Hooser et al. 1994; Mayer et al. 2002

Key Events	Evidence in Animals	Confi-dence	Key References
5. Increased plasma FSH levels from release of negative feedback of 17β –extradiol and inhibin on hypothalamus and pituitary	Long-term studies (up to one year) from 30 day treatment with either VCH (mice) or VCHD (rats)	High	Hooser et al. 1994; Mayer et al. 2002 ; Lohff et al. 2006
6. Initiation and/or promotion of ovarian tumors from increased plasma FSH levels	Cystic structures in VCH-treated mice similar to preneoplastic lesions in genetically altered mice predisposed to granulose cell tumors. Prolonged increased FSH plasma levels associated with initiation/development of ovarian tumors	Moderate	Hooser et al. 1994; Tennent et al. 1990 ; Murphy and Beamer 1973 ; Murphy 1980 ; Fuller et al. 2002

5.1.2. Is the Weight of Evidence Sufficient to Establish the MOA in Animals? The Weight of Evidence for this MOA is assessed using an approach based on the Hillcriteria for causality, originally developed for application in epidemiologic investigations(Hill, 1965).

5.1.2.1. Strength, Consistency, Specificity of Association Chronic oral exposure of female mice to VCH resulted in ovarian granulosa cell tumors(Collins et al.,1987). Preceding the tumors, a reduction in the number of follicles,particularly the primary follicles, were noted in the ovaries of female mice exposed orallyor by inhalation for 13 weeks to VCH (Collins and Manus, 1987; Bevan et al.,1996).Although it is difficult to reach any strong conclusions about the ovarian tumor incidencein rats due to poor survival in the oral chronic study, rats exposed to VCH did not showany ovarian toxicity or increased incidence of ovarian tumors (Collins and Manus, 1987;Collins et al.,1987; Bevan et al.,1996).

The species difference in ovarian toxicity appears largely due to differences in the rate of bioactivation of VCH to VCH epoxides (VCH-1,2-epoxide, VCH-7,8-epoxide and VCH-diepoxide)by cytochrome P-450 enzymes. Following treatment of female mice and ratswith a single intraperitoneal dose of VCH, only mice had detectable blood levels ofVCH-I,2-epoxide (Smith et al.,1990a). Pretreatment of VCH-dosed mice with thecytochrome P450 inhibitor chloramphenicol resulted in reduced VCH-1,2-epoxide bloodconcentrations compared to non-pretreated VCH-dosed mice (Smith et al.,1990a).

The evidence is compelling that VCH-diepoxide is the metabolite responsible forfollicular destruction. Analogues of VCH that have the potential to form only amonoepoxide metabolite failed to deplete small follicles (Hooser et al.,1993; Doerr etal.,1994), whereas compounds which can form diepoxides, such as BDandisoprene, significantly depleted follicles (Doerr et al., 1994). Indeed, the study by Smithet al.(1990b) showed thatthe potency of VCH-diepoxide to induce oocyte loss wasconsiderably greater than the monoepoxide metabolites and the parent compound VCH(Table 5). Furthermore, when male Fischer 344 rats and B6C3Fl mice were givenintraperitoneal injections of VCH, VCH-1,2-epoxide, VCH-7,8-epoxide (mice only), or VCH-diepoxide for 30 days, VCH reduced the number of small (pre-antral) oocytes inmice, whereas no detectable oocyte loss occurred in rats at the highest dose tested (Table4). However, this difference in susceptibility between mice and rats to oocyte destructiondisappeared when animals were administered VCH-diepoxide. A13-week dermal mousestudy of VCH-diepoxide has also been conducted and showed atrophy of the ovaries,characterized by a decreased number of follicles (Chhabra et al.,1990a).

Table 5
ED_{50}[a] Values for the Reduction in Small Oocyte Counts in Mice and Rats[1]

Species	VCH	VCH-1,2-epoxide	VCH-7,8-epoxide	VCH-diepoxide
Mouse	2.7	0.5	0.7	0.2
Rat	>7.4[b]	1.4	ND[c]	0.4

[1]Results from Smith *et al.* (1990b)
[a]Dose in mmnol/kg-day which reduces the small oocyte count to 50% of that observed in control animals.
[b]Highest dose given.
[c]Not determined.

The follicles that are selectively targeted by VCH-diepoxide are the primordial andprimary follicles, but not the secondary follicles (Springer et al.,1996). Results fromtime-course studies indicate that following 12 days of

		dosing with VCH-diepoxide, thereis a significant loss of primordial and primary follicles in both rats and mice, with noeffect on secondary follicle numbers (Kao et al.,1999). Longer periods of dosing (30days) with either VCH in mice or VCD in rats result in additional reduction of secondaryfollicles, but this is likely a result of a reduced population of primordial and primaryfollicles from which to recruit (Hooser et al.,1994; Flaws et al.,1994). Mechanisticstudies in rats have determined that VCH-diepoxide causes ovotoxicity by acceleratingthe natural process of atresia -which occurs through apoptosis -and this requires repeated exposures (Hoyer and Sipes, 2007). In mice dosed with VCH/VCH-diepoxide or rats dosed with VCH-diepoxide, thepathological changes in the ovary are identical (Flaws et al.,1994; Springer et al.,1996;Mayer et al.,2002). VCH-diepoxide selectively destroys the primordial and primaryfollicles, accelerating the normal process of atresia via apoptosis (Springer et al., 1996).Accelerated oocyte depletion leads eventually to premature ovarian failure and cessationof the estrous cycle. Highly elevated FSH plasma levels occur in both in rats, mice andnonhuman primates treated with either VCH (mice only) or VCH-diepoxide. At the timeof ovarian failure, VCH-treated mice showed lesions in the ovary that appear similar topreneoplastic lesions reported in a genetically susceptible strain of mice for granulosa celltumors (Hooser et al.,1994; Tennant et al.,1990). Elevated FSH levels have beenconsistently seen in various animal models of ovarian cancer and are thought to be the underlying mechanism to ovarian cancer, perhaps through alteration in signalingpathways affecting cell growth (Murphy, 1980; Fuller et al.,2002). Mice, but not rats, are susceptible to ovarian toxicity by VCH. However, a consistentassociation has been observed across species (mice, rats, and nonhuman primate)between VCH-diepoxide administration and primordial and primary follicle loss in theovary (Springer et al.,1996; Kao et al.,1999; Mayer et al.,2002; Appt et al.,2006).Unfortunately, no data are available for the tumorigenic response of VCH or VCH-diepoxideacross species. The consistent association of VCH-diepoxide exposure withfollicular loss across species, in contrast to VCH exposure where only the mouse issusceptible, can be explained by species differences in the kinetics of the metabolism ofVCH and its metabolites. The balance of activation versus detoxification reactions in ratsand mice suggest that the mouse may be more susceptible to VCH toxicity because of generation of high levels of epoxide metabolites. In general, the mouse is more efficientat metabolism of VCH to epoxides than is the rat. In contrast, the rat may be moreefficient at hydrolysis of epoxides. Thus, the rat would tend to have a lower circulatingconcentration of epoxide metabolites than the mouse at equal doses of VCH. If,however, the ultimate metabolite VCH-diepoxide is administered to either rats or mice,metabolism does not playa limiting role in the ability of the diepoxide to be formed insufficient quantity so that it can reach the ovary and target primordial and primaryfollicles. No VCH metabolism data exist for nonhuman primates. Data on olefiniccompounds, such as BD, indicate that nonhuman primates are similar tohumans with respect to cytochrome P-450 bioactivation of olefins to its epoxidemetabolites (Dahl and Henderson, 2000). Limited in vitro data with human livermicrosomes suggest that VCH metabolism in nonhuman primates is likely to be morelike the rat than the mouse (Smith and Sipes, 1991). In summary, there is strong evidence for an association of follicular loss in the mouseovary via VCH-diepoxide by a non-genotoxic pathway and the formaton of ovariantumors. The key events show strength, consistency and specificity of association. 5.1.2.2. Dose-Response Concordance The dose-response concordance between the ovarian toxicity and tumors in VCH-exposedmice cannot be evaluated. For inhalation exposure, a 13-week, but not a 2-yearchronic bioassay, was		

		conducted. The NTP conducted both 13-week and 2-year oralstudies in mice; however, only the high-dose (1200 mg/kg) female mice were evaluated for ovarian effects (follicle loss), and it is not known whether the ovarian effects were also present at the lower doses (75 to 600 mg/kg). The continuous breeding protocolstudy showed ovarian effects in mice dosed with 500 mg/kg VCH for 17 to 18 weeks;but, here again, the lower doses were not evaluated. Increased incidence of ovariantumors were seen in the 200 and 400 mg/kg dosefemale mice in the NTP chronicbioassay. Smith *et al.* (1990b) compared the dose-response relationship of the reduction in smalloocyte counts in the ovaries of mice and rats following 30 days of intraperitonealtreatment with VCH, VCH-1,2-epoxide, VCH-7,8-epoxide, and VCH-diepoxide. Thedoses of VCH and its epoxide metabolites that reduced the small oocyte count to 50%that of control are shown in the Table 5. In mice, the destruction of the small oocyteswas dependent on the administered dose of VCH. In contrast, VCH treatment producedno detectable change in oocyte number in the ovaries of rats. However, in both species,VCH mono-and di-epoxide metabolites were much more potent than the parentcompound in destroying small oocytes. The ED 50 ofVCH-1,2-epoxide, VCH-7,8-epoxideand VCH-diepoxide was 5.4-, 3.9-, and 14-fold lower than that ofVCH. The potency of VCH-diepoxide>>YCH-1,2-epoxide~VCH-7,8-epoxide>VCHin oocyte destructionwould be expected if VCH-diepoxide was the ultimate ovotoxicant. VCH-diepoxide is metabolically produced from VCH-1,2-epoxide, which in turn is metabolically formedfrom VCH. A comparison of the ED 50 of VCH between mice and rats is consistent with the susceptibility of mice to VCH-induced ovarian toxicity observed in the 13-weekinhalation and oral toxicity studies, compared to rats. This susceptibility differencedisappears when the animals are treated with VCH-diepoxide, and to a lesser extent withVCH-1,2-epoxide. Nonhuman primates given a single daily intramuscular injection of VCH-diepoxide for15 days had nearly complete elimination of primordial, intermediate, primary andsecondary follicles in the ovaries 27 days after treatment with a 250 mg/kg dose, a 50%reduction in primordial and primary follicles with 160 mg/kg, and no effect with 80mg/kg (Appt et al., 2006). No studies, however, have been conducted to determine thedose-response relationship of follicle loss and ovarian tumors in nonhuman primates. In summary, a dose-response relationship is observed in the potency of VCH and itsepoxide metabolites in inducing follicular loss in the ovary, providing strong evidence forVCH-diepoxide as the compound responsible for the ovarian toxicity, as well as thereason for the species differences in susceptibility. 5.1.2.3. Temporal Relationship A single intraperitoneal dose of 320 mg/kg VCH-diepoxide resulted in a time-dependent decrease of both primordial and small primary follicles beginning 6 days later (Devine etal., 2004). Larger follicle stages were not affected over the time period studied (12 daysfollowing dosing). The follicles that are selectively targeted by VCH-diepoxide are the primordial andprimary follicles, but not the secondary follicles (Springer et al., 1996). Results fromtime-course studies indicate that following 12 days of dosing with VCH-diepoxide, thereis a significant loss of primordial and primary follicles in both rats and mice, with noeffect on secondary follicle numbers (Kao et al., 1999). Longer periods of dosing (30days) witheither VCHin mice or VCD in rats result in additional reduction of secondaryfollicles, but this is likely a result of a reduced population of primordial and primaryfollicles from which to recruit (Hooser et al.,1994; Flaws et al., 1994). Mechanisticstudies in rats have determined that VCH-diepoxide causes ovotoxicity by acceleratingthe		

natural process of atresia -which occurs through apoptosis -and this requires repeated exposures (Hoyer and Sipes, 2007).

Ovarian failure (premature menopause) is a consequence ofVCH-induced primordial and primary follicle loss (Hooser et al., 1994; Mayer et a!., 2002). In mice given dailyintraperitoneal injections of 800 mg/kg VCH for 30 days, there was >90% loss of thesmall pre-antral follicles at the end of the dosing period (Hooser et al.,1994; Table 6).At 240 days of the study (210 days following VCH treatment), there were few widelyscattered oocytes in small and growing follicles; however, at 360 days, no oocytes at anystage were observed in the VCH-treated mice. The complete loss of oocytes at 360 dayscoincided with the loss of estrous cyclicity, indicating ovarian failure. Follicular loss alsoresulted in increased follicle stimulating hormone (FSH) plasma levels, presumably due to the lack of 17β-estradiol and inhibin production from the follicles. 17β-Estradiol and inhibin exert negative feedback inhibition of FSH production in the hypothalamus and/orpituitary. Plasma FSH levels were not elevated above control levels until 240 daysfollowing the initiation of dosing, suggesting that virtually complete loss of follicles is needed before the release of the negative feedback inhibition at the hypothalamus/pituitary.

Table 6
Long-Term Effects of 30 Days Dosing of FemaleB6C3F$_1$ Mice With 800 mg/kg VCH by Intraperitoneal Injection[1]

Day	Small follicles (% control)	Serum FSH (% above control)	Estrous cyclicity
30	11%*	30	Yes
120	3%*	50	Yes
240	<1%*	130*	Yes
360	0%*	160*	No

[1]Results from Hooser *et al.*, 1994. Day = day after onset of dosing.
*Different from controls, $p<0.05$.

A similar pattern was reported for rats dosed intraperitoneally for 30 days with 80 mg/kgVCH-diepoxide (Mayer et al., 2002). Rats dosed with VCH-diepoxide had reducednumber of preantral follicles by day 30. Following cessation of dosing, relative tocontrols, primordial, primary, and secondary follicles were progressively lost with time.Circulating FSH levels in VCH-treated rats were greater (days 120,240 and 360) than incontrols. Cyclicity was disrupted in the VCH-diepoxide treated animals by day 360.

		In the two-year NTP mouse bioassay on VCH, there were increased incidences ofuncommon ovarian tumors, including mixed benign tumors, granulosa-cell tumors, andgranulosa-cell tumors or carcinomas (combined), in female B6C3F1 mice given oraldoses ofVCH (in corn oil) for 103 weeks (Collins et al.,1987). The incidence of tubularcell or granulosa cell hyperplasia was also increased in the VCH-treated groups. Thesetumors were preceded by ovarian toxicity, characterized by a reduction in the number ofprimary follicles and mature graafian follicles, which was observed in female mice givenoral doses of VCH for 13 weeks (Collins and Manus, 1987). Likewise, in the two-year NTP dermal study on VCH-diepoxide, benign or malignantgranulosa cell tumors and or benign mixed tumors of the ovary was preceded by atrophyof the ovaries (decreased number of follicles), which was seen in the 13-week dermalstudy (Chhabra et al., 1990a,b). In summary, there is strong evidence for the temporal progression of the key events in the proposed MOA, leading to the formation of ovarian tumors. Metabolism precedes follicular loss. Complete follicular loss is required before the elevation in plasma FSHlevels and the subsequent appearance of pre-neoplastic lesions. 5.1.2.4. Biological Plausibility and Coherence Embryonic development of the ovary involves extensive proliferation of germcells andsomatic cells. In the later stages of this development, germ cells differentiate intooocytes when they cease to divide mitotically and begin to undergo meiosis (Hirshfield,1991). However, the meiotic process is not completed and oocytes are arrested in anearly stage of prophase known as the diplotene stage of meiosis (Buccione et al.,1990;Hirshfield, 1991). Somatic follicular (granulosa) cells in the embryonic ovary continue toproliferate and envelop small oocytes within a single layer to form primordial follicles(Gondos, 1970; Bacharova, 1985). Therefore, at birth, the ovary contains a finite numberof primordial follicles containing oocytes arrested in prophase of the first meiotic division(Hirshfield, 1991). A primordial follicle contains an oocyte surrounded bya single layerof fusiform-shaped granulosa cells. During follicular development, the oocyte enlargesand the granulosa cells become cubiodal in appearance to form a primary follicle. Agrowing follicle results fromproliferation of the granulosa cells into multiple layers. Allof these stages of development occur in the pre-antral stage (25-250m in diameter).Larger, more mature follicles have developed a fluid-filled antrum, and thus classified asantral follicles (>250 m in diameter). The process by which follicles are selected forovulation is not known, but the pool of available follicles is considerably greater thanthose selected for ovulation. So, there is also a gradual loss of follicles at various stagesof development byan apoptotic process called atresia. Agents that damage primordialand primary follicles to the extent of complete depletion of the available follicle poolproduce permanent infertility and premature menopause since, once destroyed, thosefollicles cannot be replaced. Several animal models initially drew attention to the possible involvement ofgonadotropins in ovarian tumorigenesis. Biskind and Biskind (1944) reported a highincidence of ovarian tumors in rats whose ovaries were autotransplanted to the spleen.However, the formation of the ovarian tumors did not occur when one ovary was leftintact or when the ovary was autotransplanted in previously hypophysectomized animals (Biskind and Biskind, 1948). This tumorigenesis has been attributed to elevated pituitarygonadotropins due to the deactivation of estrogen in the liver and the consequentdepletion of negative feedback of estrogen on the pituitary. Since then, the		

		developmentof ovarian tumors has been reported in several transgenic or knockout animal models thatexhibit hypergonadotropism with high levels of circulating FSH and LH similar to thepostmenopausal state in women (Kumar et al., 1999; Risma et al., 1995). Granulosa cell tumors can also be induced by genetic deletion of germcells (Murphy, 1972; Murphy and Beamer, 1973), neonatal thymectomy (Nishizuka et al.,1972), or X-irradiaton (Marchant,1987). The hormonal tumorigenesis hypothesis for ovarian granulosa cell cancers is thatendocrine factors that control the normal growth of target organs can also providesuitable conditions for neoplastic transformation. The gonadotropin hypothesis has beenproposed as an underlying mechanism to ovarian cancer, in that excessive levels ofgonadotropins, related to the surge occurring during ovulation and the loss of gonadalnegative feedback in menopause and premature ovarian failure (oocyte depletion), mayplaya role in the development and progression of ovarian (granulosa cell) cancer. Theincidence of ovarian cancer in women climbs dramatically around the age at which mostwomen reach menopause. The onset of menopause, which happens at approximately 51years of age, involves changes in gonadotropin levels as a result of cessation of ovarianfunction and menstrual cycle. The complete cessation of ovarian function results in theloss of negative feedback of ovarian steroids (i.e., -estradiol) on gonadotropins. In 2to 3 years after menopause, gonadotropin levels are particularly high, such that theconcentrations of FSH and LH reach a peak of 10-20 times and 3-4 times the values recorded during the proliferative phase of the menstrual cycle, respectively (Chakravartiet al., 1976; Speroff et al.,1999). The increase in plasma gonadotropin levels is a resultof the loss of feedback inhibition from 17-estradiol and inhibin, both of which areproduced from follicles. In the case of ovarian failure where there is complete loss ofoocytes in the ovary, the loss of 17-estradiol and inhibin from the follicles leads toincreased plasma gonadotropin levels. In summary, there is strong evidence of biological plausibility and coherence in theproposed MOA for mouse ovarian tumors by a non-genotoxic, threshold mechanism. 5.1.3. Are Key Events in the Animal MOA Plausible in Humans? The key events in the animal MOA are plausible in humans. VCH-diepoxide has beenshown to selectively deplete primordial and primary follicles in the ovaries of nonhumanprimates (Macaca fascicularis) (Appt et al.,2006). The physiology and anatomy ofnonhuman primates are more similar to humans than rodents. The finding that VCH-diepoxidedepletes primordial and primary follicles in nonhuman primates is strongevidence that the MOA for VCH-induced ovarian cancer is plausible in humans. Humansand nonhuman primates possess the same ability to metabolize VCH as rodents,specifically cytochrome P-450 CYP 2A, 2B and 2E1and epoxide hydrolase, as well asglutathione transferase in organs, such as the liver, lung and ovaries. Female human livermicrosomes have been shown to metabolize VCH to VCH-1,2-epoxide, but at lower ratesthan rat (2-fold) and mouse (13-fold) liver microsomes (Smith et al., 1991). 1-3-Butadiene (BD), a structural analogue of VCH, also produces ovarian atrophy(follicular loss) and ovarian tumors in mice, but not rats. The diepoxide of BD (DEB) isbelieved to be the metabolite responsible for the ovarian effects, and the speciessusceptibility is likely due to the decreased ability of the rat to produce BD diepoxide.Filser et al. (2007) was unable to detect DEB in venous blood of male Sprague-Dawleyrats (detection limit 0.01 µmol/L) exposed to 1,200 ppm for 6-8 hours, whereas DEB wasdetected in mice 3.2 µmol/Lat 1,280 ppm BD. Humans appear to be similar to rats intheir inability to produce the diepoxide metabolite. Albertini et al., (2007) reportedfindings of a molecular epidemiology study in the Czech Republic of occupationally-		

		exposedworkers with cumulative exposures up to 6.3 ppm-weeks. Any N,N-(2,3-dihydroxy-1,4-butadiyl) valine (pry-Val) hemoglobin adduct of DEB that may have beenpresent in these workers were below the limit of detection of the assay used. Swenberg etal. (2007) compared results in the Czech Republic occupationally-exposed workers toresults in mice and rats for a pry-Val adduct at similar BD concentrations. Itwasconcluded that production of DEB in humans is below levels produced in both mice andrats exposed to as little as 1 ppm BD by inhalation. Subsequently, Georgieva et al.(2007) reported in anabstract that these adducts were detected at a low concentrations inCzech Republic workers when a more sensitive analytical method to measure pry-Valadducts was used. There was, however, no clear dose-response relationship between pry-Valadducts and BD concentrations, indicating that pry-Val adducts may be formed fromother unknown sources besides the BD in the workplace environment. Thus, using BD as an analogy, it is possible that VCHmay be metabolized in human toVCH diepoxide in humans; but as is the case with BD, at extremely low levels whencompared to the mouse. 5.1.4. Taking into Account Kinetic and Dynamic Factors, is the Animal MOA Plausible in Humans? The diepoxide appears to be the metabolite of VCHresponsible for the specific targeted destruction of promordial and primary follicles. There are species differences in the ratesof formation or activation of the epoxide metabolites of VCH, as well as in the rate of detoxification. Mice have significantly greater capacity to metabolize VCH to the mono-anddi-epoxides than do rats, and in many cases performs the reactions more efficientlythan rats. In particular, mouse liver and lung tissue are very active in their ability tometabolize VCHto the epoxide metabolites. In contrast, the mouse does not hydrolyzeepoxides well, while the rat hydrolyzes the epoxides to a greater extent than the mouse.This balance of activation reactions with detoxification reactions leads to the conclusionthat the mouse may be more susceptible to the toxic effects of VCH, since the VCHdiepoxide is considered the metabolite responsible for follicular destruction in the ovary.The prediction that VCHepoxidation rate in the liver and lung is the major factor whichdetermines the ovotoxicity and carcinogenesis of VCHis supported by the toxicity data.Ovarian effects are only observed in mice exposed to VCHeither orally or by inhalation,with tumors seen in mice dosed orally with VCH. Further support of the role of metabolism in the susceptiblity of animal species to the ovotoxicity of VCH comes fromstudies which show that the rat develops follicular loss and, ultimately ovarian failure, ifdosed with VCH-diepoxide. Thus, if the epoxidation rate of VCH is the critical factorwhich determines the ovotoxicity and carcinogenicity of VCH, then the rat would be themore appropriate animal model for extrapolation of the VCHanimal data to humans.Based on the available in vitro human liver microsomes data, human metabolism ofVCHis expected to be more similar to the rat than the mouse. Given thatthe rat did notdevelop ovarian effects (follicular loss) either from oral or inhalation exposure, humanswould also not be expected to develop ovotoxicity, and thus would not be expected todevelop ovarian tumors at or below the exposures used in these animal studies. References from Sapphire (2008) Albertini, R.J., Sram, R.J., Vacek, P.M., Lynch, .I., Rossner, P., Nicklas, J.A., McDonald, J.A., Boysen, G., Georgieva, N., and Swenberg, J.A. (2007) Molecular epidemiological studies in 1,3-butadiene exposedCzech workers: female-male comparisons. Chem. Biol. Int. 166: 63-77 Appt, S.E., Kaplan, I.R., Clarkson, T.B., Cline, I.M., Christian, and Hoyer, P.B (2006) Destruction of primordial		

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Carcinogenicity

Mutagenicity

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
06/07/2011	United States / Individual	Pages 33-35. Section 4.9.1.2 In vivo data and Table 12 I was involved with the mouse and rat micronucleus assays as an industry representative in a consortia (my employer at the time was Exxon Biomedical Sciences, Inc.). I am also the lead author of the published paper. The CLH report states that there were no concurrent positive controls in the mouse micronucleus studies by Bevan et al. (2001). This, however, is incorrect. 1,3-Butadiene was used as the positive control for the mouse studies. For both the 2-day and the 13-week inhalation studies, concurrent groups of mice were exposed to 1,000 ppm 1,3-butadiene so that a comparison could be made between the two compounds. 1,3-Butadiene is a suitable positive control for mice since in a number of independent studies on 1,3-butadiene, positive results have been reported for the bone marrow and peripheral blood micronucleus assay in mice (EU Risk Assessment Report for 1,3-Butadiene, Volume 20). In the VCH mouse micronucleus assays, the 1,3-butadiene-exposed mice showed significantly more MN-PCEs than the control animals. Bevan, C., Keller, D.A, Pinepinto, A.S., and Bentley, K.S. (2001) Effect of 4-vinylcyclohexene on micronucleus formation in the bone marrow of rats and mice. Drug Chemical Toxicol. 24: 273-285.	Thank you for this information. However, for regulatory purposes, a positive control substance should be validated. Data on historical positive/negative controls would be helpful.	We agree with MSCA

Toxicity to reproduction

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
13/07/2011	Sweden / Member State	We also agree with the submitting MS that the screening study show effects on testicular sperm concentration and oocyte/follicles without apparently impacting fertility. With the carcinogenic effect in the ovary and the lack of data on reproduction KemI think that it is important that more data is gathered for evaluation of the reproductive effects of VCH.	Thanks for your support. We agree that reprotoxic endpoint should be evaluated during substance evaluation.	The need for more information is noted. However substance evaluation is not within the portfolio of RAC.
06/07/2011	United States / Christopher Bevan / Individual	*ECHA comment: The document attached "Bevan C., 2009., ADDITIONAL COMMENTS ON THE CLH REPORT ON 4-VINYLCYCLOHEXENE (VCH), (Comments on CLH Report on 4-VCH cjb.docx)is copied below:* ADDITIONAL COMMENTS ON THE CLH REPORT ON 4-VINYLCYCLOHEXENE (VCH) Provided by Dr. Christopher Bevan, PhD, DABT, atoxicology consultant and managing principal of CJB Consulting LLC	Thanks for these interesting data on the MOA of VCH and its potential for endocrine disruption. The CLH report has	Noted. See also comments from Dr. Bevan in page 32, and our response.

<table>
<tr><th>Date</th><th>Country / Organisation / MSCA</th><th>Comment</th><th>Dossier submitter's response to comment</th><th>RAC's response to comment</th></tr>
<tr><td></td><td></td><td>
A proposed mode of action (MOA) for the ovarian tumors in mice from VCH (Bevan et al., 2009).

Following exposure and uptake, VCH is metabolized, primarily in the liver, to VCH-1,2-epoxide or VCH-7,8-epoxide, which are further metabolized to VCH-diepoxide. VCH-diepoxide enters the blood and circulates through the body. Upon reaching the ovary, VCH-diepoxide selectively destroys the primordial and primary follicles through a mechanism involving programmed cell death or apoptosis. Repeated exposures to VCH ultimately result in premature ovarian failure, due to complete follicular loss. Since 17β-estradiol and inhibin are no longer produced from the primordial and primary follicles in the ovary, loss of the negative feedback inhibition of follicle-stimulating hormone (FSH) release from the hypothalamus and pituitary occurs, leading to high plasma levels of FSH. Increased plasma levels of FSH result in the initiation and/or promotion of ovarian tumors.

Bevan, C., Gargas, M., Kirman, C., and Vergnes, J. (2009) Mode of action (MOA) evaluation and derivation of a cancer and non-cancer reference value for 4-vinylcyclohexene. Toxicol. Sci. 108 (Suppl.), abstract #839

Key Events in the Proposed MOA for VCH-Induced Ovarian Tumors
<table>
<tr><th>Key Events</th><th>Evidence in Animals</th><th>Confidence</th><th>Key references</th></tr>
<tr><td>1. Systemic levels of VCHD</td><td>Blood levels inferred from studies showing blood levels of VCH-1,2-epoxide in VCH-dosed mice, and from toxicity studies with VCHD.</td><td>High</td><td>Smith et al., 1990a,b,c; Keller et al., 1997</td></tr>
<tr><td>1a. Bioactivation of VCH to VCHD (via VCH-1,2-epoxide)</td><td>In vitro liver, lung and ovary microsome studies., with formation greater in mice than rats. Inhibition of cytochrome P450 reduces VCH-1,2-epoxide formation in vivo and in vitro</td><td>High</td><td></td></tr>
</table>
</td><td>been revised to provide more information on this MOA, based on your communication and on the review from Hoyer and Sipes (2007).</td><td></td></tr>
</table>

Date	Country / Organisation / MSCA	Comment				Dossier submitter's response to comment	RAC's response to comment
		1b. Hydrolysis of VCH epoxide metabolites by epoxide hydrolase	In vitro liver, lung and ovary microsome studies, with rats having higher epoxide hydrolase rates than mice.	High			
		2. Increased follicular loss in ovaries from VCHD	VCHD more potent ovotoxicant than VCH monoepoxides and VCH; VCH analogue studies show ovotoxicity only from compounds producing the diepoxide; subchronic/chronic mouse studies of VCHD show same ovarian effects and tumors as with VCH; rats/mice dosed with VCHD show identical ovarian effects as with mice dosed with VCH.	High	Smith *et al.*, 1990b; Hooser *et al.*, 1993; Doerr *et al.*, 1995; Chhabra *et al.*, 1990a,b; Flaws *et al.*, 1994 ; Bevan *et al.*, 1996; Collins and Manus, 1987 ; Collins *et al.*, 1987		
		3. Selective destruction of primordial and primary follicles through apoptosis	Secondary follicles not directly affected by VCHD treatment; morphological and biochemical pathway studies.	High	Hooser *et al.*, 1994; Flaws *et al.*, 1994; Springer *et al.*, 1996; Kao *et al.*, 1999; Mayer *et al.*, 2002; Hu *et al.*, 2001a,b; 2002		
		4. Ovarian failure (no estrous cyclicity) from complete oocyte loss	Long-term studies (up to one year) from 30 day treatment with either VCH (mice) or VCHD (rats);	High	Hooser *et al.*, 1994; Mayer *et al.*, 2002		
		5. Increased plasma FSH levels from release of negative feedback of 17β-estradiol and inhibin	Long-term studies (up to one year) from 30 day treatment with either VCH (mice) or VCHD	High	Hooser *et al.*, 1994; Mayer *et al.*, 2002; Lohff *et al.*, 2006		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment

on hypothalamus/pituitary	(rats)		
6. Initiation and/or promotion of ovarian tumors from increased plasma FSH levels	Cystic structures in VCH-treated mice similar to preneoplastic lesions in genetically altered mice predisposed to granulosa cell tumors. Prolonged increased FSH plasmal levels associated with initiation/development of ovarian tumors.	Moderate	Hooser *et al.*, 1994; Tennent *et al.*, 1990; Murphy and Beamer, 1973; Murphy, 1980; Fuller *et al.*, 2002

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Collins, J.J., and Manus, A.G. (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. Health 21: 493-505.

Collins, J.J., Montali, R.J., and Manus, A.G. (1987) Toxicological evaluation of 4-vinylcyclohexene: II. Induction of ovarian tumors in female B6C3F1 mice by chronic oral administration of 4-vinylcyclohexnene. J. Toxicol. Environ. Health 21: 507-524.

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Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Flaws, J.A., Doerr, J.K., Sipes, I.G., and Hoyer, P.B. (1994) Destruction of pre-antral follicles in adult rats by 4-vinylcyclohexene diepoxide. Reprod. Toxicol. 8: 509-514. Fuller, P.J., Chu, S., Fikret, S., and Burger, H.G. (2002) Molecular pathogenesis of granulosa cell tumours. Mol. Cell. Endocrinol. 191: 89-96. Hooser, S.B., Parola, L.R., Van Ert, M.D., and Sipes, I.G. (1993) Differential ovotoxicity of 4-vinylcyclohexene and its analog, 4-phenylcyclohexene. Toxicol. Appl. Pharmacol. 119: 302-305. Hooser, S.B., Douds, D.P., DeMerell, D.G., Hoyer, P.B., and Sipes, I.G. (1994) Long-term ovarian and gonadotropin changes in mice exposed to 4-vinylcyclohexene. Reprod. Toxicol. 8: 315-323. Hu, X., Christian, P., Thompson, K.E., Sipes, I.G., and Hoyer, P.B. (2001a) Apoptosis induced by rats by 4-vinylcyclohexene diepoxide is associated with activation of the caspase cascades. Biol. Reprod. 65: 87-93. Hu, X., Christian, P., Sipes, I.G., and Hoyer, P.B. (2001b) Expression and redistribution of cellular Bad, Bax and Bcl-x(l) protein is associated with VCD-associated ovotoxicity in rats. Biol. Reprod. 65: 1489-1495. Hu, E., Flaws, J.A., Sipes, I.G., and Hoyer, P.B. (2002) Activation of mitogen-activated protein kinases and AP-1 transcription factor in ovotoxicity induced by 4-vinylcyclohexene diepoxide in rats. Biol. Reprod. 67: 718-724. Keller, D.A., Carpenter, S.C., Cagen, S.Z., and Reitman, F.A. (1997) In vitro metabolism of 4-vinylcyclohexene in rat and mouse liver, lung, and ovary. Toxicol. Appl. Pharmacol. 144: 36-44. Kao, S.-W., Sipes, I.G., and Hoyer, P.B. (1999) Early effects of ovotoxicity induced by 4-vinylcyclohexene tdiepoxide in rats and mice. Reprod. Toxicol. 13: 67-75. Lohff, J.C., Christian, P.J., Marion, S.L., and Hoyer, P.B. (2006) Effect of duration of dosing on onset of ovarian failure in a chemical-induced mouse model of perimenopause. Menopause 13: 482-488. Mayer, L.P., Pearsall, P.J., Christian, P.J., Devine, P.J., Payne, C.M., McCuskey, M.K., Marion, S.L., Sipes, I.G., and Hoyer, P.B. (2002) Long-term effects of ovarian follicular depletion in rats by 4-vinylcyclohexene dipoxide. Reprod. Toxicol. 16: 775-781. Murphy, E.D. (1980) Major experimental models of ovarian tumors: histogenesis and evaluation. In: Biology of ovarian neoplasia (E.D. Murphy and W.G. Beamer, Eds), UICC Technical Report Series 50: 66-73.		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Murphy, E.D., and Beamer, W.G. (1973) Plasma gonadotropin levels during early stages of ovarian tumorigenesis in mice of the Wx/Wv genotype. Cancer Res. 33: 721-723. Nishizuka, Y., Tanaka, Y., Sakakura, T., and Kojima, A. (1972) A frequent development of ovarian tumors from dysgenetic ovaries of neonatally thymectomized mice. Gann 63: 139-140. Smith, B.J., Carter, D.E., and Sipes, I.G. (1990a) Comparison of the disposition and in vitro metabolism of 4-vinylcyclohexene in the female mouse and rat. Toxicol. Appl. Pharmacol. 105: 364-371. Smith, B.J., Mattison, D.R., and Sipes, I.G. (1990b) The role of epoxidation in 4-vinylcyclohexene-induced ovarian toxicity. Toxicol. Appl. Pharmacol. 105: 372-381. Smith, B.J., Sipes, I.G., Stevens, J.C., and Halpert, J.R. (1990c) The biochemical basis for the species difference in hepatic microsomal 4-vinylcyclohexene epoxidation between female mice and rats. Carcinogenesis 11: 1951-1957. Springer, L.N., McAsey, M.E., Flaws, J.A., Tilly, J.L., Sipes, I.G., and Hoyer, P.B. (1996) Involvement of apoptosis in 4-vinylcyclohexene diepoxide-induced ovotoxicity in rats. Toxicol. Appl. Pharmacol. 139: 394-401. Tennent, B.J., Shultz, K.L., Sundberg, J.P., and Beamer, W.G. (1990) Ovarian granulosa cell tumorigenesis in SWR-derived F1 hybrid mice: Preneoplastic follicular abnormality and malignant disease progression. Am. J. Obstet. Gynecol. 163: 625-634.		

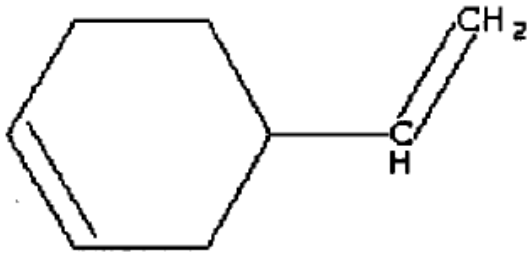
Respiratory sensitisation

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		No comments		

Other hazards and endpoints

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
29/06/2011	Spain/ Member State	With respect to the information found (this information is attached), this substance should be classified for environment as N R51/53 S60/61 according to Directive 67/548/EEC and as Chronic 2 according to CLP Regulation based on the surrogate system. The substance is not ready degradable (OECD 301C 0% degradation on 28 days) and a Daphnia EC50 (48h) of 1.9 mg/L, since not chronic experimental data are available for all trophic levels. In the information found all aquatic toxicity data have assigned a Klimisch score of 2, due to these values were found in a Japanese data base, but these are support by ECOSAR predictions although the chemical category assigned by the program was neutral organic.	Cf rules detailed in Art.36 of the CLP regulation	Noted. RAC only assess effects proposed for harmonisation for VCH by the dossier submitter (MSCA).
	The 4-Vinylcyclohexene Group comprised of ExxonMobil and INVISTA	*ECHA comment: The document attached "Synthetic Organic Chemical Manufacturers Association (SOCMA) 4-Vinylcyclohexene Work Group, 2006, 4-Vinylcyclohexene Group – Robust Summary and Test Plan, Chemical Abstracts Service Registry Number: 100-40-3, Washington DC (100-40-3 Robust summary.pdf)* 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) 72 1-4 100. Sincerely, Richard E. Opatick 4-VCH Group, Director cc: 4-VCH Membership Enclosure - CD of Robust Summary and Test Plan 4-Vinylcyclohexene Chemical Abstracts Service Registry Number: 100-40-3	From this IUCLID, only repeated toxicity, mutagenicity and carcinogenicity data have been re-examined as we consider that they are the only appropriate endpoints regarding our proposal. Then a mouse lymphoma assay performed by the NTP is now included in the revised version of the CLH report	Noted

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		 U.S. EPA HPV Challenge Program Submission Submitted by: Synthetic Organic Chemical Manufacturers Association (SOCMA) 4-Vinylcyclohexene Work Group Prepared by: Experien Health Sciences, Inc. 6322 Water Point Court Kingwood, Texas 77346 28 1 -8 1 2-6667 **Table of Contents** **1. PLAIN LANGUAGE SUMMARY.....3** **2. CHEMICAL DESCRIPTION.....5** **3. PRODUCTION, USE AND EXPOSURES.....5** 3.1. PRODUCTION AND USE.....5 3.2. DIRECT WORKER EXPOSURES.....5 3.3. INDIRECT WORKER EXPOSURES.....6 3.4. INDIRECT CONSUMER EXPOSURES.....6 3.5. RELEASES TO THE ENVIRONMENT.....6 **4. PYSICOCHEMICAL PROPERTIES.....7**		

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		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.		

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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.

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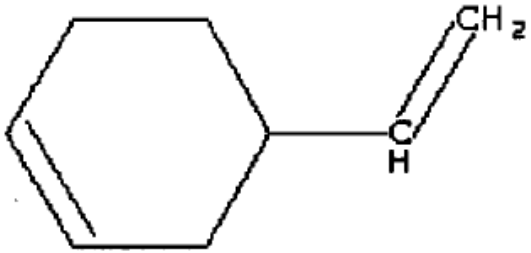
		2. CHEMICAL DESCRIPTION 4-Vinylcyclohexene (4-VCH, CASRN 100-40-3), a dimer of 1,3-butadiene, is a colorless liquid with the following chemical structure:  Molecular Formula: C8-H12 Molecular Weight: 108.18 4-VCH can be sold commercially at ≥ 97% pure. 4-VCH sold at high purity typically contains approximately 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		

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		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		

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		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:		

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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.

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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:

<table>
<tr><th>Reaction</th><th>Conc. of Sensitizer (molecules/cm^3)</th><th>Rate Constant (cm3/molecule-sec)</th><th>Est. Half-Life (hours)</th><th>Rel†</th><th>Source</th></tr>
<tr><td>Ozone</td><td>7×10^{11}</td><td>21.2×10^{-17}</td><td>1.3</td><td>2</td><td>Modeled</td></tr>
<tr><td>OH-</td><td>1.5×10^{6}</td><td>89.3×10^{12}</td><td>1.4</td><td>2</td><td>Modeled</td></tr>
</table>
† 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
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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	LEVEL 1 version 3.00 Fugacity-based model

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			Melting Point: -108.9°C	
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*,

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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)

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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

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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 (≥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,

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		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 $\geq$ 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)		

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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)

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		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)

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		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. <u>Reproductive and Developmental Toxicity</u> 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		

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		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. <u>Metabolism and Toxicokinetics (non-SIDS Endpoint)</u> 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).		

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		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.		

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		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): Flagg: 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		

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		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:		

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		- 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		

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		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/cm³ **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		

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		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**		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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:		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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**		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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:		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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.		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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		

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		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 %		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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.		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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.		

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		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.		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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)		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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:**		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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**		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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,		

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		2, 4 or 6 hr post-dose, blood (cardiac puncture) collected into heparinised 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		

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		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.		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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		

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		(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 4-VCH 1,2-EP (mg/kg bw) (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 4-VCH 1,2-EP		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		(mg/kg bw) (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		

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		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		

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		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		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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		

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		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		

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		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		

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		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 ------		

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		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		

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		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.		

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		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		

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		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		

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		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 -- --		

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		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		

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		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		

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		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		

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		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		

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		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		

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		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.		

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		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.		

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		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)		

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		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		

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		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		

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		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		

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		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		

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		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.		

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		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		

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		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)		

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		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.		

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		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		

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		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		

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		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%		

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		- 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		

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		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.		

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		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		

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		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		

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		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		

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		(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 =		

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		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%		

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		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		

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		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		

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		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		

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		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.		

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		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.		

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		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.		

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		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		

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		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		

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		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		

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		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		

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		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		

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		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. Sex N Mean(%) 95% Conf. Limits (ppm) 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		

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		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.		

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		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		

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		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		

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		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		

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		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.]		

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		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		

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		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		

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		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),		

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		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		

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		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		

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		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		

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		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		

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		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		

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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

Parameter	--- mg/kg bwt/day ---	
	0	500
Mating index^	16/20	18/20
Fertility index^^	19/20	19/20

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		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		

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		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		

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		(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		

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		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: Small oocyte count Day (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% *		

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		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		

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		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%		

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		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		

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		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.		

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		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,		

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		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		

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		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		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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)		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		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.		

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Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		2. HAZARDS IDENTIFICATION **Classification of the substance or mixture** **REGULATION (EC) No 1272/2008**		

Skin Corrosion / irritation	Category 2
Carcinogenicity	Category 2
Chronic aquatic toxicity	Category 3
Flammable liquids.	Category 2

Classification according to EU Directives 67/548/EEC or 1999/45/EC
For the full text of the R phrases mentioned in this Section, see Section 16
Symbol(s) F - Highly flammable
Xn - Harmful
R -phrase(s) R11 - Highly flammable
R38 - Irritating to skin
R40 - Limited evidence of a carcinogenic effect
Risk Combination Phrases R52/53 - Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment

2. HAZARDS IDENTIFICATION

Label Elements

<table>
<tr><th>Date</th><th>Country / Organisation / MSCA</th><th>Comment</th><th>Dossier submitter's response to comment</th><th>RAC's response to comment</th></tr>
<tr><td></td><td></td><td>
Signal Word Danger
Hazard Statements
H315 - Causes skin irritation
H351 - Suspected of causing cancer
H412 - Harmful to aquatic life with long lasting effects
H225 - Highly flammable liquid and vapor
Precautionary Statements - EU (§28, 1272/2008)
P281 - Use personal protective equipment as required
P273 - Avoid release to the environment
P302+ P352 - IF ON SKIN: Wash with plenty of soap and water
P210 - Keep away from heat/sparks/open flames/hot surfaces. - No smoking
P240 - Ground/Bond container and receiving equipment
Other Hazards
No information available.

<h2>3. COMPOSITION/INFORMATION ON INGREDIENTS</h2>
<table>
<tr><th>Component</th><th>EC No.</th><th>Weight %</th><th>CAS-No</th><th>Classification</th><th>GHSCLAS</th><th>REACH Reg. No.</th></tr>
<tr><td>4-Vinylcyclohexene
100-40-3</td><td>EEC No. 202-848-9</td><td>99</td><td>100-40-3</td><td>F; R11
Xi; R38
Carc. Cat. 3; R40
R52/53;</td><td>Flam. Liq. 2 (H225)
Skin Irrit. 2 (H315)
Carc. 2 (H351)
Aquatic Chronic 3 (H412)</td><td></td></tr>
</table>
For the full text of the R phrases mentioned in this Section, see Section 16

<h2>4. FIRST AID MEASURES</h2>
Description of first aid measures
Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes Obtain medical attention
Skin Contact Wash off immediately with soap and plenty of water removing all contaminated clothes and shoes Obtain medical attention
Ingestion Clean mouth with water Get medical attention
Inhalation Remove from exposure, lie down Move to fresh air
Notes to Physician Treat symptomatically
<h2>5. FIRE-FIGHTING MEASURES</h2>
Extinguishing media
Suitable Extinguishing Media
Cool closed containers exposed to fire with water spray Water spray Carbon dioxide (CO_2) Dry chemical chemical
</td><td></td><td></td></tr>
</table>

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		foam **Extinguishing media which must not be used for safety reasons** No information available. **Special hazards arising from the substance or mixture** Flammable Vapors may travel to source of ignition and flash back **Advice for fire-fighters** As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear **6. ACCIDENTAL RELEASE MEASURES** **Personal precautions, protective equipment and emergency procedures** Ensure adequate ventilation **Environmental precautions** Prevent further leakage or spillage if safe to do so **Methods and material for containment and cleaning up** Soak up with inert absorbent material (e.g. sand, silica gel, acid binder, universal binder, sawdust). Keep in suitable and closed containers for disposal. Remove all sources of ignition. Use spark-proof tools and explosion-proof equipment. **7. HANDLING AND STORAGE** **Precautions for Safe Handling** Avoid contact with skin and eyes Do not breathe dust Do not breathe vapors or spray mist Use only in area provided with appropriate exhaust ventilation Use explosion-proof equipment Use only non-sparking tools **Conditions for safe storage, including any incompatibilities** Keep in a dry place Keep container tightly closed Keep away from heat and sources of ignition Refrigerator/flammables Keep under nitrogen **Specific End Uses** **8. EXPOSURE CONTROLS / PERSONAL PROTECTI** **Control parameters** **Exposure limits** **Component** 4-Vinylcyclohexene European Union: — The United Kingdom: — France: — Belgium: TWA: 0.1 ppm; TWA: 0.45 mg/m³ Spain: VLA-ED: 0.1 ppm; VLA-ED: 0.45 mg/m³ **Component** 4-Vinylcyclohexene Italy: — Portugal: TWA: 0.1 ppm The Netherlands: — Finland: — Denmark: TWA: 0.4 mg/m³; TWA: 0.1 ppm **Component** 4-Vinylcyclohexene Austria: — Switzerland: MAK: 0.1 ppm Poland: NDS: 10 mg/m³ Norway: — Ireland: TWA: 0.1 ppm; TWA: 0.4 mg/m³		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Derived No Effect Level (DNEL) No information available. **Predicted No Effect Concentration (PNEC)** No information available. **Exposure controls** **Engineering Measures** Use explosion-proof electrical/ventilating/lighting/equipment Ensure that eyewash stations and safety showers are close to the workstation location **Personal protective equipment** **Eye Protection** Goggles **Hand Protection** Protective gloves **Skin and body protection** Wear appropriate protective gloves and clothing to prevent skin exposure **Respiratory Protection** Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced **Hygiene Measures** Handle in accordance with good industrial hygiene and safety practice **Environmental exposure controls** No information available. **9. PHYSICAL AND CHEMICAL PROPERTIES** **Physical State** Liquid **Appearance** Clear **odor** odorless **pH** No information available. **Vapor Pressure** 15 mbar @ 20 °C **Vapor Density** 3.76 **Viscosity** 0.7 mPa s at 20 °C **Boiling Point/Range** 126 - 127°C / 258.8 - 260.6°F@ 760 mmHg **Melting Point/Range** -101°C / -149.8°F **Flash Point** 16°C / 60.8°F **Explosion Limits** **Lower** 0.6 **Upper** 9.1 **Water Solubility** 0.05 g/L (20°C) **Specific Gravity** 0.832 **Molecular Formula** C8 H12 **Molecular Weight** 108.18 **10. STABILITY AND REACTIVITY** **Reactivity** **Chemical Stability**		

<table>
<tr><th>Date</th><th>Country / Organisation / MSCA</th><th>Comment</th><th>Dossier submitter's response to comment</th><th>RAC's response to comment</th></tr>
<tr><td></td><td></td><td>
Stable under normal conditions
Possibility of Hazardous Reactions
Hazardous Polymerization Hazardous polymerization does not occur.
Hazardous Reactions . No information available.
Conditions to Avoid
Keep away from open flames, hot surfaces and sources of ignition, Excess heat, Incompatible products.
Incompatible Materials
Strong oxidizing agents, Alcohols, Amines.
Hazardous Decomposition Products
Carbon monoxide (CO). Carbon dioxide (CO_2).

11. TOXICOLOGICAL INFORMATION
Information on Toxicological Effects
<u>Acute Toxicity</u>
Product Information

Component Information
<table>
<tr><td>Component</td><th>LD50 Oral</th><th>LD50 Dermal</th><th>LC50 Inhalation</th></tr>
<tr><td>4-Vinylcyclohexene</td><td>3080 µL/kg (Rat)</td><td>20 mL/kg (Rabbit)</td><td></td></tr>
</table>
<u>Chronic Toxicity</u>
Carcinogenicity

The table below indicates whether each agency has listed any ingredient as a carcinogen
<table>
<tr><td>Component</td><th>IARC</th><td>UK</td></tr>
<tr><td>4-Vinylcyclohexene</td><td>Group 2B</td><td></td></tr>
</table>
Sensitization No information available.
Mutagenic Effects No information available
Reproductive Effects No information available.
Developmental Effects No information available.
Target Organs No information available.
Other Adverse Effects The toxicological properties have not been fully investigated. See actual entry in RTECS for
complete information
Endocrine Disruptor Information None known
12. ECOLOGICAL INFORMATION
<u>Toxicity</u>
Ecotoxicity effects
Do not empty into drains
</td><td></td><td></td></tr>
</table>

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment

Component	Freshwater Algae	Freshwater Fish	Microtox	Water Flea
4-Vinylcyclohexene		Oncorhynchus mykiss: LC50=17 mg/L 48h		>100 mg/L 48h

Persistence and degradability
Not readily biodegradable

Bioaccumulative potential
No information available.

Mobility in soil
No information available.

Results of PBT and vPvB assessment
Other adverse effects
No information available

13. DISPOSAL CONSIDERATIONS

Waste treatment methods
Waste from Residues / Unused Products Dispose of in accordance with local regulations
Contaminated Packaging Empty containers should be taken for local recycling, recovery or waste disposal

14. TRANSPORT INFORMATION

IMDG/IMO
UN-No 1993
Hazard Class 3
Packing Group II
Proper Shipping Name Flammable liquid, n.o.s.

ADR
UN-No 1993
Hazard Class 3
Packing Group II
Proper Shipping Name FLAMMABLE LIQUID, N.O.S.

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		IATA **UN-No** 1993 **Hazard Class** 3 **Packing Group** II **Proper Shipping Name** FLAMMABLE LIQUID, N.O.S.* **15. REGULATORY INFORMATION** **Safety, health and environmental regulations/legislation specific for the substance or mixture** **International Inventories** Component: 4-Vinylcyclohexene; EINECS: 202-848-9; ELINCS: -; NLP: ; TSCA: X; DSL: X; NDSL: -; PICCS: X; ENCS: X; CHINA: X; AICS: X; KECL: KE-35356 X **Legend** **TSCA** - United States Toxic Substances Control Act Section 8(b) Inventory **EINECS/ELINCS** - European Inventory Lists **DSL/NDSL** - Canadian Domestic Substances List/Non-Domestic Substances List **PICCS** - Philippines Inventory of Chemicals and Chemical Substances **ENCS** - Japan Existing and New Chemical Substances **CHINA** - China Inventory of Existing Chemical Substances **AICS** - Inventory of Chemical Substances **KECL** - Existing and Evaluated Chemical Substances **Chemical Safety Assessment** **16. OTHER INFORMATION** **Text of R phrases mentioned in Section 2-3** R11 - Highly flammable R38 - Irritating to skin R40 - Limited evidence of a carcinogenic effect R52/53 - Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment **Revision Date** 10-Nov-2010 **Revision Summary** Not applicable **This safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006** **Disclaimer** **The information provided on this SDS is correct to the best of our knowledge, information and belief at the date of its** **publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation,** **disposal and release and is not to be considered as a warranty or quality specification. The information relates only to the** **specific material designated and may not be valid for such material used in combination with any other**		

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		material or in any process, unless specified in the text End of Safety Data Sheet		

ATTACHMENTS RECEIVED:

General Comments

Comment to the French proposal for Harmonized Classification and Labeling of 4-Vinylcyclohexene (CAS 100-40-3), (**BASF_CLH_100-40-3.pdf**) – Submitted by Germany / AffiliatedWithOrganisation / Company-Manufacturer

Letter from Cefic, PlasticsEurope and SRP, 12/07//2011, *4-vinyl cyclohexene (VCH) Response to proposal for harmonised classification and labelling* (**CEFIC Letter re 4VCH.pdf**) - Submitted by Belgium / Graeme Wallace / Cefic / BehalfOfAnOrganisation / Industry or trade association

Carcinogenicity

4-vinyl cyclohexene (VCH) Response to proposal for harmonised classification and labelling, (**VCH-Comments.docx**) – Submitted by Belgium / Graeme Wallace / Cefic / BehalfOfAnOrganisation / Industry or trade association

Comments from Evonik Industries, 12/07/2011, (**evonik_statement_CLH_VCH_France.pdf**) – Submitted by Germany / BehalfOfAnOrganisation / Company-Manufacturer

Toxicity to Reproduction

Bevan C., 2009., *ADDITIONAL COMMENTS ON THE CLH REPORT ON 4-VINYLCYCLOHEXENE (VCH),* (**Comments on CLH Report on 4-VCH cjb.docx**) – Submitted by United States / Christopher Bevan / Individual

Other Hazards and Endpoints

Synthetic Organic Chemical Manufacturers Association (SOCMA), 4-Vinylcyclohexene Work Group, 2006, *4-Vinylcyclohexene Group – Robust Summary and Test Plan*, Chemical Abstracts Service Registry Number: 100-40-3, Washington DC (**100-40-3 Robust summary.pdf**) – Submitted by Spain / Manuel Carbo / Member State

Experien Health Sciences Inc., 2006, *IUCLID Data Set*, (**100-40 3 IUCLID 4.pdf**) – Submitted by Spain / Manuel Carbo / Member State

Acros Organics, 10/11/2010, *Safety Data Sheet*, (**100-40 3 SDS.pdf**) - Submitted by Spain / Manuel Carbo / Member State