Committee for Risk Assessment

RAC

Annex 1

Background document

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

1,1-dichloroethylene; vinylidene chloride

EC Number: 200-864-0
CAS Number: 75-35-4

CLH-O-0000007324-77-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

8 June 2023

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Chemical name: 1,1-dichloroethylene; vinylidene chloride

EC Number: 200-864-0

CAS Number: 75-35-4

Index Number: 602-025-00-8

Contact details for dossier submitter:

ANSES (on behalf of the French MSCA)

14 rue Pierre Marie Curie

F-94701 Maisons-Alfort Cedex

classification.clp@anses.fr

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,1-DICHLOROETHYLENE; VINYLIDENE CHLORIDE

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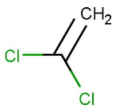
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,1-DICHLOROETHYLENE; VINYLIDENE CHLORIDE

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name in the IUPAC nomenclature	1,1-dichloroethene
Other names (usual name, trade name, abbreviation)	1,1-dichloroethylene ethene, 1,1-dichloro- ethylene, 1,1-dichloro- vinylidene chloride vinylidene dichloride VDC 1,1-DCE
EC number (if available and appropriate)	200-864-0
EC name (if available and appropriate)	1,1-dichloroethylene
CAS number (if available)	75-35-4
Other identity code (if available)	FDA UNII: 21SK105J9D UN 1303 NCI-C54262 InChi: 1S/C2H2Cl2/c1-2(3)4/h1H2 InChi Key: LGXVIGDEPROXKC-UHFFFAOYSA-N Compound CID: 6366
Molecular formula	C2H2Cl2
Structural formula	
SMILES notation (if available)	ClC(=C)Cl
Molecular weight	96.94 g/mol
Degree of purity (%) (if relevant for the entry in Annex VI)	/

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self- classification and labelling (CLP)
1,1-dichloroethylene CAS 75-35-4	**≥ 99 and ≤ 100 % (w/w)**	Flam. Liq. 1; H224 Carc. 2; H351 Acute Tox. 4 *; H332	Flam. Liq. 1; H224 Flam. Gas. 1; H220 Acute Tox. 4; H332 Acute Tox. 4; H302 Acute Tox. 3; H331 Acute Tox 3; H301 Skin Irrit. 2; H315 Eye irrit. 2; H319 Eye irrit. 2A, H319 Carc. 1B (inhalation); H350 Carc. 2; H351 STOT RE 1 (nose; inhalation); H372 STOT RE 2 (liver; oral); H373 STOT RE 2 (liver; inhalation, oral); H373 STOT RE 2 (not reported; dermal, oral); H373 Aquatic chronic 2; H411 Aquatic chronic 3; H412

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
/				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self- classification and labelling (CLP)	The additive contributes to the classification and labelling
See confidential Annex					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	602-025-00-8	1,1-dichloroethylene; vinylidene chloride	200-864-0	75-35-4	Flam. Liq. 1 Carc. 2 Acute Tox. 4*	H224 H351 H332	GHS02 GHS08 GHS07 Dgr	H224 H351 H332			D
Dossier submitters proposal	602-025-00-8	1,1-dichloroethylene; vinylidene chloride	200-864-0	75-35-4	**Retain** Flam. Liq. 1 **Modify** Carc. 1B Acute Tox. 1 **Add** Muta. 2 Acute Tox. 3 STOT RE 1 Aquatic Chronic 3	**Retain** H224 **Modify** H350 H330 **Add** H341 H301 H372 (liver, kidney, respiratory tract) H412	**Retain** GHS02 GHS08 Dgr **Add** GHS06 **Remove:** GHS07	**Retain** H224 **Modify** H350 H330 **Add** H341 H301 H372 (liver, kidney, respiratory tract) H412		**Add** inhalation: ATE = 0.5 mg/L (dusts or mists) oral: ATE = 200 mg/kg bw	**Retain** D
Resulting entry in Annex VI if adopted by RAC and agreed by Commission	602-025-00-8	1,1-dichloroethylene; vinylidene chloride	200-864-0	75-35-4	Flam. Liq. 1 Carc. 1B Muta. 2 Acute Tox. 1 Acute Tox. 3 STOT RE 1 Aquatic Chronic 3	H224 H350 H341 H330 H301 H372 (liver, kidney, respiratory tract) H412	GHS02 GHS08 GHS06 Dgr	H224 H350 H341 H330 H301 H372 (liver, kidney, respiratory tract) H412		inhalation: ATE = 0.5 mg/L (dusts or mists) oral: ATE = 200 mg/kg bw	D

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	Harmonised classification proposed Acute Tox 3 – H301 ATE = 200 mg/kg bw	Yes
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Harmonised classification proposed Acute Tox 1 – H330 ATE = 0.5 mg/L	Yes
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class assessed in this dossier Data inconclusive	Yes
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	Harmonised classification proposed: Muta 2 – H341	Yes
Carcinogenicity	Harmonised classification proposed: Carc. 1B – H350	Yes
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Harmonised classification proposed STOT-RE 1 (liver, kidney, respiratory tract) – H372	Yes
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Harmonised classification proposed: Aquatic chronic 3 – H412	Yes
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

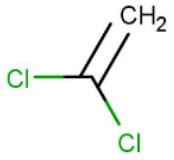
The current classification of 1,1-dichloroethylene (or vinylidene chloride; VDC) is old and was published on the Adaptation to Technical Progress 00 (ATP 00) from the previous European directive 67/548/CEE:

- Flam. Liq. 1 – H224
- Acute Tox. 4* - H332
- Carc. 2 – H351
- Note D

This current harmonised classification is based on data available at the time.

New data was generated on VDC. A major piece of new information was the NTP Technical Report carried out by National Toxicology Program (NTP, National Institutes of Health, U.S. Department of Health and Human Services) published in 2015 and a recent new *in vivo* Comet assay (2016).

In the ECHA's letter to Registrants about the "Notification of information obtained and conclusion made after completion of dossier evaluation" dated on 7 February 2017, ECHA said "taking into account the information in the updated dossier, ECHA considers the substance as a possible candidate for a proposal for harmonised classification and labelling according to Article 37 of Regulation (EC) No 1272/2008…". In this context, lead registrants contacted France in order to submit a proposal for an update of the current harmonised classification. Relative to the current harmonised classification, several hazard classifications have been proposed to be changed or added.

RAC general comment
1,1-Dichloroethene, commonly called 1,1-dichloroethylene or vinylidene chloride (VDC), is a colourless liquid with a mild, sweet smell. It is an organochloride with the molecular formula $C_2H_2Cl_2$ and has the structure shown below. Like most chlorocarbons, VDC is poorly soluble in water, but soluble in organic solvents. In the absence of an added inhibitor, such as monomethyl ether of hydroquinone, vinylidene chloride readily polymerises. In the presence of air or oxygen, shock-sensitive and explosive peroxides are formed.    VDC is a man-made chemical that is not known to occur naturally. It is produced commercially via the dehydrochlorination of 1,1,2-trichloroethane in the presence of an

aqueous alkali, like sodium hydroxide or lime. Vinylidene chloride can be purified through distillation and extraction.

VDC is used as an intermediate in organic synthesis reactions and in the production of a monomer in a variety of polymers. Several end products are made of VDC polymers, such as food plastic wrap, carpet latex backing, fire- and ignition-resistant clothing, vapour barriers for insulation, steel pipe coating, outdoor furniture, paper and board coatings, adhesives, and photographic film.

The substance is registered under the REACH Regulation and is manufactured in and/or imported to the European Economic Area at ≤ 1000 to < 10 000 tonnes per year.

VDC is restricted under the REACH regulation and listed in the Annex XVII (entry 38). The conditions of restrictions state that 1,1-Dichloroethylene "shall not be placed on the market, or used, as substance or as constituent of other substances, or in mixtures in concentrations equal to or greater than 0,1 % by weight, where the substance or mixture is intended for supply to the general public and/or is intended for diffusive applications, such as in surface cleaning and cleaning of fabrics".

The current classification of VDC is published on the Adaptation to Technical Progress 00 (ATP 00) and originated from the previous European directive 67/548/CEE:

- Flam. Liq. 1 – H224
- Acute Tox. 4* - H332
- Carc. 2 – H351
- Note D: "Certain substances which are susceptible to spontaneous polymerisation or decomposition are generally placed on the market in a stabilised form……"

The current harmonised classification is based on data available at the time. Since then, new data has been generated which have been reviewed by the dossier submitter (DS). The main data sources for the CLH dossier as well as for the current RAC opinion are:

- NTP 2015
- IARC 2019
- Comet assay (Anonymous, 2016)

The DS, considering the new generated data as well as the self-classifications proposed in the C&L Inventory, justified that action is needed at a Community level and the following endpoints are assessed in the CLH report:

- Acute toxicity (oral and inhalation)
- Serious eye damage/eye irritation
- Germ cell mutagenicity
- Carcinogenicity
- Specific target organ toxicity — repeated exposure
- Short-term (acute) aquatic hazard
- Long-term (chronic) aquatic hazard

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] No requirement for justification for the CMR classification

The update of the CMR classification (from H351, Carc. 2 to H350, Carc. 1B and new proposal as Muta. 2 – H341) does not need to be justified.

[B.] Justification for the following hazard classes assessed in the current CLH report:

- Acute Tox (oral): Differences in self-classification
    - Acute Tox 3 – H301: 43/612 notifiers
    - Acute Tox 4 – H302: 47/612 notifiers

- Acute tox (inhalation): Change in existing entry due to new interpretation/evaluation of existing data

- STOT RE : Differences in self-classification
    - STOT RE1 – H372 (nose; inhalation): 39/612 notifiers
    - STOT RE2 – H373 (liver; oral): 39/612 notifiers
    - STOT RE2 – H373 (not reported; dermal, oral): 6/612 notifiers
    - STOT RE2 – H373 (not reported): 1/612 notifiers
    - STOT RE2 – H373 (liver; inhalation, oral): 1/612 notifiers

- Aquatic chronic: Differences in self-classification
    - Aquatic chronic 2 – H411: 21/612 notifiers
    - Aquatic chronic 3 – H412: 109/612 notifiers

5 IDENTIFIED USES

The substance is registered under the REACH Regulation and is manufactured in and/or imported to the European Economic Area at ≥ 1000 to < 10 000 tonnes per annum.

According to the lead registrants, VDC is an industrial chemical, used as an intermediate in organic synthesis reactions and as a monomer in the production of a variety of polyvinylidene chloride copolymers.
Theses copolymers of vinylidene chloride have a broad spectrum of applications in the plastic industry and the major application is the production of films for food packaging. They are also used in many types of packing materials, as flame retardant coatings for fiber and carpet backing, in piping, as coating for steel pipes and in adhesive applications.

This substance is restricted under REACH regulation and listed in the Annex XVII (entry 38). The conditions of restrictions state that 1,1-Dichloroethylene “shall not be placed on the market, or used, as substance or as constituent of other substances, or in mixtures in concentrations equal to or greater than 0,1 % by weight, where the substance or mixture is intended for supply to the general public and/or is intended for diffusive applications such as in surface cleaning and cleaning of fabrics”.

6 DATA SOURCES

In 2019, a new comprehensive literature search over the period 2010 – March 2019 was performed by the lead registrants using SciFinder® (from American Chemical Society) and based on the CAS number. The data search done before 2010 in the context of the REACH registration dossier could not be retrieved.

In addition, the dossier submitter has based its evaluation on the information provided in the REACH registration dossier and an additional bibliographic search (done up to the 05/08/2021 for human health properties and up to beginning of 2021 for environmental properties).

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	liquid at 20°C and 101.3 kPa	References cited in ECHA website: Merck index, 1989 Patty's Toxicology, 1994 Hawley's Condensed Chemical Dictionary, 1997 Kirk-Othmer Encyclopedia of Chemical Technology, 2002	
Melting/freezing point	150.5K at 101.3 kPa = -123.15°C at 101.3 kPa	ECHA website	The melting point reported in different data sources ranges from -122.5 to -122.6°C.
Boiling point	304.6K at 101.3 kPa = 30.85°C at 101.3 kPa	ECHA website	The reported boiling points range between 31.56 and 31.7°C
Relative density	1.215 at 20°C	ECHA website	The reported densities range between to 1.21 and 1.22 g/m^3
Vapour pressure	66340 Pa at 20°C	ECHA website	
Surface tension	Waived	/	study scientifically not necessary (surface activity is not a desired property of the material)
Water solubility	2.5 g/L at 21°C	ECHA website	
Partition coefficient n-octanol/water	Log Kow (Log Pow): 2.13 at 20°C	ECHA website	Except for the calculated estimate provided in the Verschueren Handbook of Environmental data on Organic Chemicals, all values provided by the reliable sources where in a narrow range, i.e. between 2.02 to 2.13. As a conservative approach, a log Pow of 2.13 is defined as the key parameter.
Flash point	245K (-28°C) at 1013 hPa	ECHA website	The flash point of VDC was rather consistent: -28°C (closed cup method) and between -30 and -16°C (open cup method).

Property	Value	Reference	Comment (e.g. measured or estimated)
Flammability	extremely flammable	ECHA website	Reported flammability limits were all between 5.6 % for the lower limit and 16 % for the upper limit
Explosive properties	Waived	/	study scientifically not necessary (no chemical groups present in the molecule which are associated with explosive properties)
Self-ignition temperature	786K at 1013 hPa	ECHA website	
Oxidising properties	Waived	/	study scientifically not necessary (the substance is flammable)
Granulometry	Not applicable	/	study scientifically not necessary (the substance is marketed or used in a non solid form)
Stability in organic solvents and identity of relevant degradation products	Waived	/	study scientifically not necessary (stability not considered critical)
Dissociation constant	Waived	/	study scientifically not necessary (the substance is an organic molecule without ionisable groups)
Viscosity	0.358mPa.s (dynamic) at 20°C	ECHA website	According to the consulted data sources, the viscosity of VDC ranged between 0.33 to 0.4485 mPa s. The median of this range (N=4) was defined as the key parameter, i.e. 0.358 mPa s (Lange's Handbook of Chemistry).

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Toxicokinetics data of VDC have been reviewed in the recent NTP (2015) and IARC (2019) reports and summarised below. References can be found in the source documents.

Absorption

Following inhalation exposure in rats, the absorption of vinylidene chloride was rapid and concentration-dependent. The uptake was linear for concentrations up to 150 ppm, above which the uptake decreased with increasing concentration. The compound was found in blood within 2 minutes following exposure. Following oral administration of doses ranging from 0.5 to 100 mg/kg, vinylidene chloride was rapidly and almost completely absorbed in rats and mice and distributed to all tissues examined. Peak blood levels were observed in rats within 2 to 8 minutes.

Distribution

Following inhalation exposure to concentrations up to 2,000 ppm [^{14}C]vinylidene chloride, the highest level of total radioactivity was found in the liver and kidney, with only very small amounts present in other tissues. Covalently bound radioactivity was also highest in the liver and the kidney (fasted rats having higher levels than nonfasted). Following exposure to 10 ppm for 6 hours, a higher body burden was observed in mice compared to rats exposed under similar conditions. The bound radioactivity was higher in mouse liver and kidney than in corresponding tissues in rats.

Vinylidene chloride was distributed to all tissues following oral administration with the highest amount found in the liver and kidney.

Metabolism

The proposed pathway for the metabolism of vinylidene chloride in rodents is shown in Figure 1. Vinylidene chloride is metabolized in rodents via pathways involving CYP2E1 to yield three reactive metabolites: vinylidene chloride epoxide, 2-chloroacetyl chloride and 2,2-dichloroacetaldehyde. These electrophilic metabolites undergo oxidation, hydrolysis and reactions with glutathione and cellular macromolecules. Relatively high levels of CYP2E1 are present in three primary target organs of vinylidene chloride in rodents: liver, kidney, and lung.

The involvement of glutathione in the detoxification of vinylidene chloride was consistent with the observation that exposure to vinylidene chloride depletes liver glutathione levels. Urinary metabolites identified were N-acetyl-S-(2-hydroxyethyl)cysteine, S-(cysteinyl acetyl) glutathione, N-acetyl-S-(2-carboxymethyl) cysteine, thiodiglycolic acid, dithioglycolic acid, dithiodiglycolic acid and chloroacetic acid. Biliary metabolites identified were S-(2-carboxymethyl) glutathione, S-(cysteinyl acetyl)glutathione and a product of the intramolecular rearrangement of the metabolite, S-(2-chloroacetyl)glutathione. In addition, several carboxymethylated proteins were identified in the bile from vinylidene chloride-treated rats. Mice metabolized a greater portion of the orally administered vinylidene chloride than rats. Although the types of metabolites observed in rats and mice were similar, N-acetyl-S-(2-carboxymethyl)cysteine arising likely from the 2-chloroacetyl chloride pathway was detected in mice but not in rats. In addition, quantitatively, mice produced more S-(2-hydroxyethyl)-N-acetyl cysteine, a product of the reaction between vinylidene chloride epoxide with glutathione, than rats suggesting that the formation of vinylidene chloride epoxide is higher in mice than in rats.

In addition, several investigations performed on rat liver microsome incubations and mouse liver and lung microsomal incubations have shown that vinylidene chloride epoxide is the major and likely the most important cytotoxic metabolite; minor metabolites identified were 2,2,-dichloroacetaldehyde and 2-chloroacetylchloride. As seen *in vivo*, these metabolites undergo secondary reactions including oxidation, glutathione conjugation and hydrolysis. The levels of the acetal observed in lung microsomes were higher than those in the liver microsomal incubations. It was also demonstrated that the mean rate of formation of the epoxide was two-fold higher in mouse lung microsomal incubations compared to human's ones. Both CYP2E1 and CYP2F2 catalyze the bioactivation of vinylidene chloride to its epoxide in the mouse lung microsomes. Using incubations of mouse lung microsomes, and recombinant CYP2E1 (rat and human), CYP2F2 (mouse), CYP2F3 (goat) and CYP2F4 (rat), it was further demonstrated that vinylidene chloride metabolism occurred with different affinities and catalytic efficiencies in different species, suggesting species differences in the severities of toxicities by vinylidene chloride. Recombinant rat CYP2E1 showed greater affinity and efficiency for vinylidene chloride than human CYP2E1, mouse CYP2F2, goat CYP2F3 or rat CYP2F4.

There are several critical factors that contribute to the metabolism of vinylidene chloride. Glutathione levels and glutathione S-transferase activity, nutritional status (fasting and nonfasting) and changes in CYP2E1 are important factors. Inducers and inhibitors of CYP2E1 alter metabolic activation of vinylidene chloride to reactive intermediates. In rodents, vinylidene chloride epoxide and 2-chloroacetylchloride are proposed as the reactive intermediates produced in the liver following exposure which are subsequently detoxified via the reaction with glutathione. These electrophilic intermediates are also capable of reacting with cellular macromolecules to form adducts in the liver, which may partially explain the observed liver toxicity in rodents. The glutathione conjugates are secreted from the hepatocytes and delivered to the kidney where they undergo glomerular filtration. In the kidney, glutathione conjugates formed in the liver may be metabolized to the corresponding cysteine conjugate, which is acetylated and excreted in urine. Alternately, glutathione conjugates can be metabolized by β-lyase, an enzyme located in the renal proximal tubule, to release an electrophilic product that can subsequently interact with cellular macromolecules in the kidney. This mechanism has been shown to be associated with the observed nephrotoxicity of other halogenated ethylenes and ethanes. It has been shown that fasting (inducing GSH depletion) significantly reduces detoxification and enhances covalent binding of toxic metabolites in the liver and kidney.

Elimination

Elimination of vinylidene chloride following inhalation exposure in rats was rapid with the majority of the dose eliminated in the urine. Steady state levels in expired air were achieved following exposure to 25 to 150 ppm vinylidene chloride, indicating that the elimination is first order at these levels; about 1% of the dose was excreted unchanged in the expired air at these exposure concentrations. At concentrations greater than 150 ppm, levels in expired air increased indicating saturation of metabolism. The pulmonary elimination was biphasic in rats following inhalation exposure; the half-lives for the first and second phases, respectively,

based on the unchanged compound were 20 and 217 minutes following exposure to 10 ppm and 21 and 133 minutes following exposure to 200 ppm [14C]vinylidene chloride. Urinary elimination followed a similar pattern; the half-lives for the first and second phases, respectively, based on the total [14C] excretion in urine were 3.1 and 19.3 hours following exposure to 10 ppm and 3.8 and 23.9 hours following exposure to 200 ppm [14C]vinylidene chloride. The major portion of the dose was eliminated in both the breath and the urine during the rapid first phase. Limited data in mice following inhalation exposure to 10 ppm vinylidene chloride indicated that the elimination of unchanged compound in the expired air is smaller and elimination via urine is larger compared to rats, indicating that mice metabolize vinylidene chloride at a greater rate than rats.

An investigation of the plasma toxicokinetics of vinylidene chloride in Sprague Dawley rats showed that the Cmax and AUC0–∞ following inhalation exposure to 300 ppm were respectively 2.8 mg/L and 279 μg·min/mL; the elimination half-life and bioavailability were respectively 50 minutes and 55.7%.

Following oral administration, the pattern of elimination was similar to that following inhalation exposure. Following a single administration of 1 mg/kg in rats, about 1% to 3% of the dose was excreted in expired air as unchanged chemical, with 21% recovered as carbon dioxide. The majority of the dose was eliminated in urine (63%) and some in feces (16%) within 72 hours, with the majority excreted within the first 24 hours. Following administration of 50 mg/kg, 16% to 30% of the dose was excreted in expired air as the parent with concomitant reductions in the expired carbon dioxide (3% to 6%) and urinary excretion (35% to 47%) suggesting that metabolism saturates at rather low doses. Mice eliminated less in expired air as unchanged chemical and more in urine than rats following oral administration of 50 mg/kg. The elimination of vinylidene chloride following oral administration in rats was biphasic. Half-lives for pulmonary elimination were, respectively for the two phases, 25 and 117 minutes for a 1 mg/kg dose and 21 and 66 minutes for 50 mg/kg. For urinary elimination of total radioactivity, the estimated half-lives for the first and second phases were 6 and 17 hours for both doses. Plasma toxicokinetics of vinylidene chloride in Sprague Dawley rats following gavage exposure showed a similar behavior to inhalation exposure. The Cmax and AUC0–∞ following gavage exposure to 30 mg/kg were 8.9 mg/L and 233 μg·min/mL, respectively; the elimination half-life and bioavailability were 88 minutes and 46.5%, respectively.

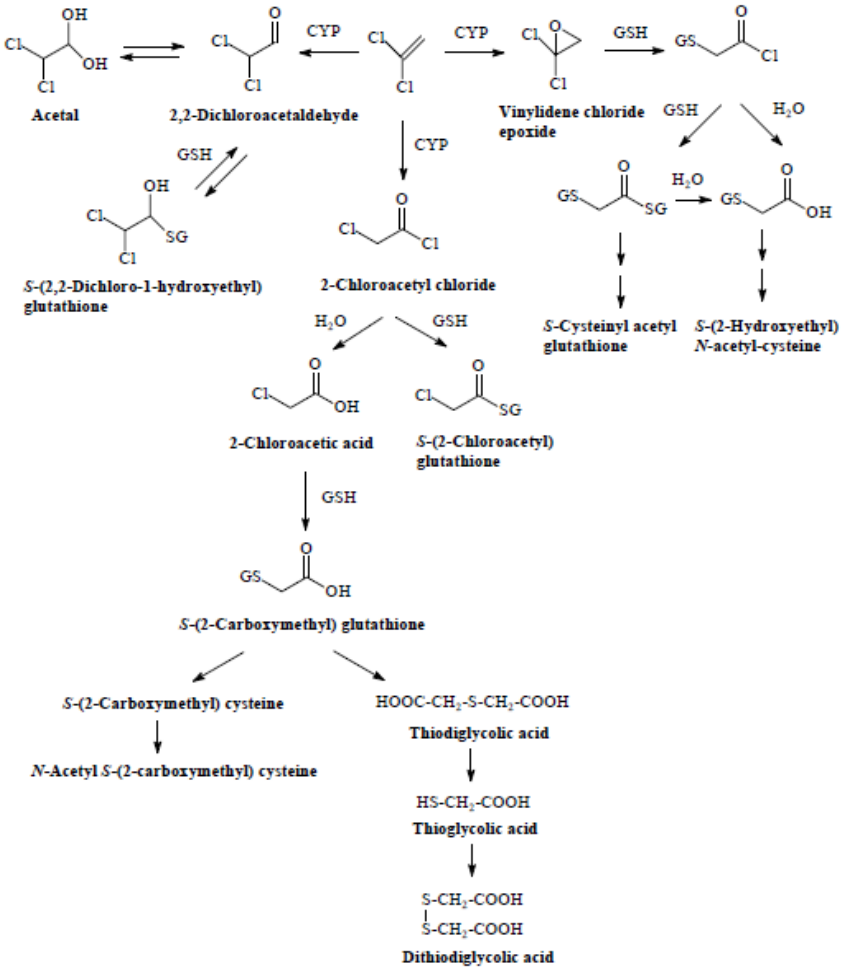


Figure 1: Proposed Metabolic Pathway of Vinylidene Chloride in Rodents (from IARC, 2019)

RAC evaluation of available information on toxicokinetics

The toxicokinetic data on VDC has been reviewed in the recent U.S. National Toxicology Program (NTP, 2015) and the International Agency for Research on Cancer (IARC, 2019) reports and was summarised by the DS.

Exposure to VDC predominantly takes place via the oral and inhalation route. The latter is regarded as the main one, since VDC is a very volatile liquid with a boiling point of 30.85°C at 1 atm and a high vapour pressure (66340 Pa at 20°C). Because of its low relative molecular mass and hydrophobic nature, dermal absorption is also likely, but there are no relevant published data. The chemistry of the VDC, its reaction products and metabolites as well as the toxicokinetic information about their distribution and/or persistency at specific sites/organs after exposure, are important aspects in the evaluation of the toxicity of VDC. Available data come mainly from studies on rodents.

Absorption

VDC is well absorbed from the lungs and gastrointestinal (GI) tract as it is a small,

uncharged, lipophilic molecule. Following inhalation exposure in rats, the absorption of VDC was rapid and concentration dependent. The uptake was linear for concentrations up to 150 ppm, above which the uptake decreased with the increasing concentration. The compound was found in blood of rats within 2 minutes following exposure.

Following oral administration of doses ranging from 0.5 to 100 mg/kg bw, VDC was rapidly and almost completely absorbed in rats and mice and distributed to all tissues examined. Peak blood levels were observed in rats within 2 to 8 minutes.

The administration of equivalent oral and inhaled doses to rats results in significantly higher arterial blood levels and nephrotoxicity in animals inhaling the chemical.

Distribution

Although VDC is rapidly distributed to all tissues examined following either oral or inhalation exposure, most of the free VDC, its metabolites, and covalently bound derivatives are found in the liver and kidney. Following inhalation exposure to [^{14}C] VDC, the highest level of total radioactivity was found in the liver and kidney, with only small amounts present in other tissues. Covalently bound radioactivity was also highest in the liver and the kidney, where fasted rats presented higher levels than non-fasted. Mice were found to accumulate more VDC compared to rats (liver and kidney) under similar inhalation exposure conditions.

Following oral administration, VDC was rapidly and almost completely absorbed in rats and mice and distributed to all examined tissues with the highest amount found in the liver and kidney.

Metabolism

The proposed metabolic pathways of VDC in rodents are shown in the figure below. Cytochrome P450 (CYP) 2E1 is the predominant enzyme responsible for the oxidation of VDC. CYP2E1 metabolises VDC to three reactive electrophilic metabolites: vinylidene chloride epoxide (1), which is the major and likely the most important cytotoxic metabolite, 2-chloroacetyl chloride (2) and 2,2-dichloroacetaldehyde (3). These metabolites undergo oxidation, hydrolysis and reactions with glutathione (GSH) and other cellular macromolecules. Relatively high levels of CYP2E1 are present in rodents' liver, which is the most important site of VDC biotransformation, as well as in kidney and lung. Mechanistic studies using inducers and inhibitors of CYP2E1, or agents that deplete hepatic GSH levels, demonstrate the important role of biotransformation of VDC. The enzyme inducers enhance both the metabolic activation of VDC and cytotoxicity, while certain inhibitors decrease its biotransformation and toxicity. Detoxification of VDC by GSH is consistent with the observation that exposure to VDC depletes liver GSH levels. The highest CYP2E1 content is observed in centrilobular hepatocytes, followed by bronchiolar Clara cells and renal proximal tubular cells in the mouse.

In vitro studies have also shown that levels of acetal produced via metabolite (3) in lung microsomes were higher than those in the liver.

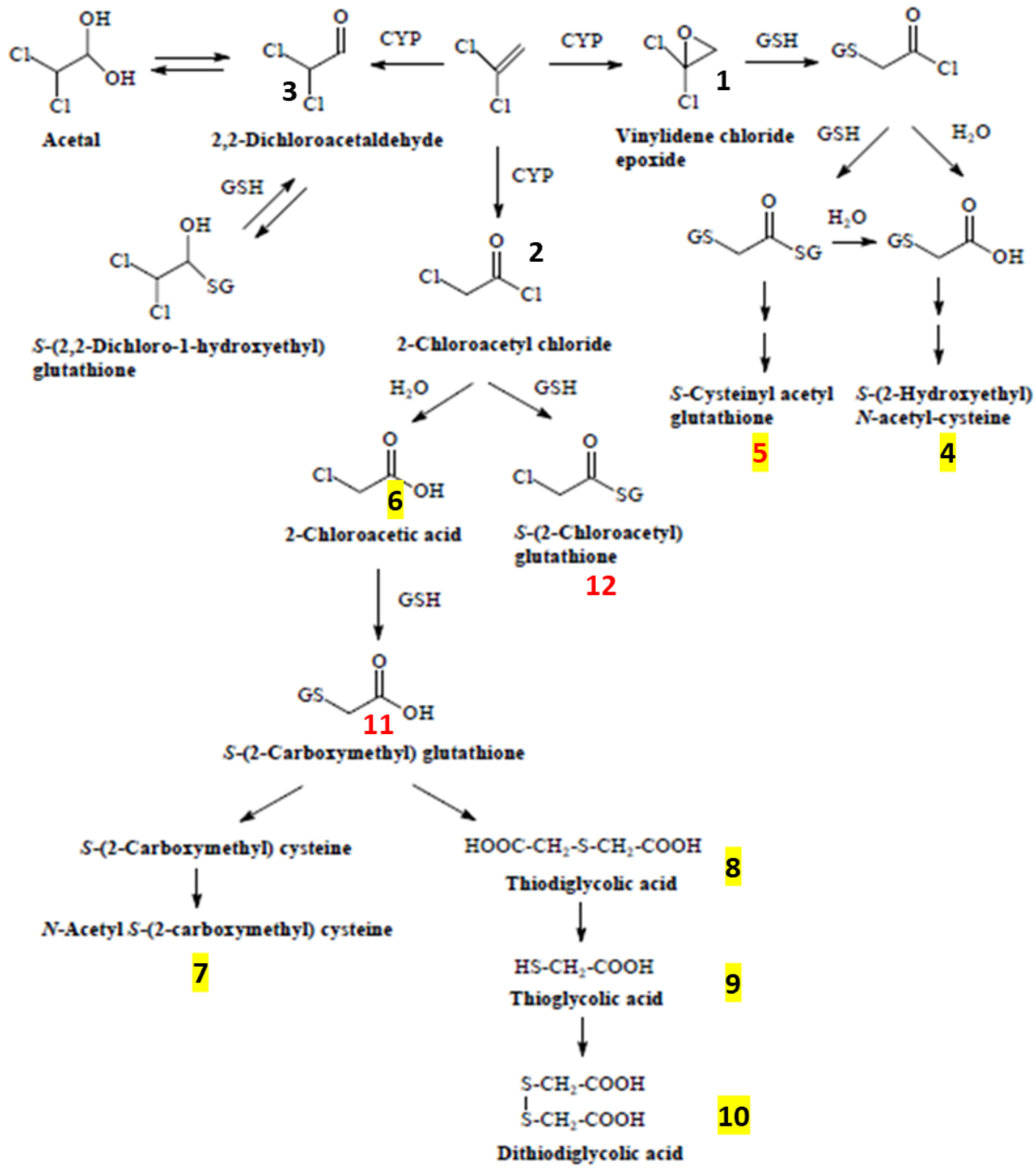


Figure: Proposed metabolic pathways of VDC in rodents (*in vivo* and *in vitro* data). Urine metabolites are highlighted with yellow colour; red colour represents biliary metabolites.

There are several critical factors that contribute to the metabolism of VDC. GSH levels and glutathione S-transferase activity, nutritional status (fasting and non-fasting) and changes in CYP2E1 are important parameters in the metabolic pathway of VDC. It has been shown that fasting (inducing GSH depletion) significantly reduces detoxification and enhances covalent binding of toxic metabolites in the liver and kidney. VDC metabolism occurs with different affinities and catalytic efficiencies in different species, suggesting species differences in the severities of toxicities by VDC. Humans appear to be less sensitive, as the mean rate of formation of the epoxide was two-fold higher in mouse lung and liver microsomal incubations compared to those from humans. In addition, renal CYP2E1 activity in humans is very low.

The rate of formation of vinylidene chloride epoxide (1) and 2,2-dichloroacetaldehyde (3) was much lower in human lung and liver microsomes compared with that of mouse. Rats and mice present a similar metabolic profile qualitatively, but mice have increased metabolic output compared to rats regarding orally administered VDC. The formation of vinylidene chloride epoxide (1) is expected to be higher in mice than in rats, since metabolite (4), which is a product of the reaction between metabolite (1) with GSH, is more abundant in mice than rats.

Sex differences have also been observed; VDC metabolism by kidney microsomes from male mice was six times greater than that from females. Sex differences were also observed in the CYP2E1-catalysed metabolic activation of VDC in mouse lung microsomes with the following order: adult female > weanling male = weanling female > adult male.

Elimination

The primary route for elimination of unchanged VDC is exhalation, while the primary route for elimination of metabolites is urinary excretion. Following inhalation exposure in rats, elimination of VDC was rapid, with most of the dose eliminated in the urine. Steady state levels in expired air were achieved following VDC exposure at 25 to 150 ppm with about 1% of the dose excreted unchanged in the expired air, indicating that the elimination is first order at these levels. At concentrations greater than 150 ppm, levels in expired air increased indicating saturation of metabolism. Pulmonary elimination was biphasic in rats after inhalation exposure, as a result of the lipophilicity of VDC. A 2-compartment model was followed, where the substance equilibrates between blood and the adipose tissue, for example. The half-lives for the first and second phases, respectively, based on the unchanged compound, were 20 and 217 minutes, respectively, following exposure to 10 ppm. When exposure to [^{14}C]VDC raised to 200 ppm, half-lives of 21 and 133 minutes were observed. Urinary elimination followed a similar pattern; the half-lives for the first and second phases, respectively, based on the total [^{14}C] excretion in urine, were 3.1 and 19.3 hours following exposure to 10 ppm and 3.8 and 23.9 hours following exposure to 200 ppm [^{14}C]VDC. During the rapid first phase, elimination mainly occurred via breathing and urine. Limited data in mice following inhalation exposure to 10 ppm VDC indicated that the elimination of unchanged compound in the expired air was smaller and elimination via urine was larger compared to rats, possibly due to a greater rate of metabolism in mice compared to rats.

Following oral administration, the pattern of elimination was similar to that following inhalation exposure. After a single administration of 1 mg/kg bw in rats, about 1% to 3% of the dose was excreted in expired air as parent compound. The majority of the dose was eliminated in urine (63%) and some in faeces (16%) within 72 hours, with the majority excreted within the first 24 hours. Following administration of 50 mg/kg bw, excretion of the parent compound in expired air increased to 16% - 30% of the dose, suggesting that metabolism saturates at rather low doses. Mice eliminated less in expired air as unchanged chemical and more in urine than rats following oral administration of 50 mg/kg bw in accordance with the inhalation results. The elimination, both pulmonary and urine, of VDC following oral administration in rats was also biphasic.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity

10.1.1 Acute toxicity - oral route

Table 8: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Gavage Vehicle: corn oil Observation period: 14 days	Mice B6C3F1, male and female 5 animals/sex/dose	Vinylidene chloride Purity: 99 %	0, 10, 50, 100, 500 and 1000 mg/kg bw	50 mg/kg bw < LD50 < 500 mg/kg bw Acute Tox. 3 or 4	NTP, 1982 Klimisch 1 Key study
	Rat Fischer 344, male and female 5 animals/sex/dose			LD50 > 1000 mg/kg bw Acute Tox. 4 or no classification	
Gavage Vehicle: corn oil	Swiss OF, mice (IFFA-CREDO), Male Total of 40 animals (10/group)	Vinylidene chloride Purity: 99 %	200 mg/kg bw	LD50 > 200 mg/kg bw Acute Tox. 3 or 4 or no classification	Ban M. et al., 1995 Klimisch 2
Gavage Vehicle: corn oil	Holtzman male rats Number of animals/group not provided	Vinylidene chloride Purity not provided	Not specified	LD50 = 1510 mg/kg bw Acute Tox. 4	Jenkins L.J. et al., 1972 Klimisch 3
Gavage Vehicle: olive oil	Inbred BDIV rats 4 animals/sex/dose	Vinylidene chloride Purity: 99 %	Not specified	LD50 = 1800 mg/kg bw for males, 1500 mg/kg bw for females. Acute Tox. 4	Ponomarkov V et al., 1980 Klimisch 3
Intragastric Vehicle: corn oil	Alderley Park male and female mice 6 animals/sex/dose	Vinylidene chloride Purity not provided	5 groups, doses not specified	LD50 male = 217 mg/kg bw LD50 female = 194 mg/kg bw Acute Tox. 3	Jones B.K. et al., 1978a Klimisch 4
Oral, not further specified	Rat, strain and sex not specified	Vinylidene chloride Purity not provided	Not specified	LD50 = 2500 mg/kg bw No classification	Kennedy G.L. et al., 1991 Klimisch 4

10.1.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

A single-dose range-finding study was conducted by the NTP (1982) as a preliminary assessment of toxicity aiming at studying the carcinogenic potency of vinylidene chloride. This acute study was performed to determine the doses to be used for a 14-day repeated-dose study. Therefore, as the derivation of a LD50 was not the purpose of the assay, the authors did not derive one.

Vinylidene chloride was diluted in corn oil and administered in a single dose by gavage to groups of five males and five females of mice and rats at 0, 10, 50, 100, 500 and 1000 mg/kg bw. The animals were observed for 14 days and then killed and necropsied on day 15.

Concerning mice, no mortality was observed at 10 and 100 mg/kg bw, 1/5 female died at 50 mg/kg bw, 5/5 males and 3/5 females died at 500 mg/kg bw. No survival of either male or female was observed at 1000 mg/kg bw. The LD50 is considered between 50 mg/kg bw (males: no lethality, females: 20 % lethality) and 500 mg/kg bw (males: 100% lethality, females: 60% lethality).

Concerning rats, no mortality was observed at 50 and 100 mg/kg bw. One female rat receiving 500 mg/kg bw died. One male rat receiving 10 mg/kg bw died after 7 days and two male rats receiving 1000 mg/kg bw died within 48 hours. The LD50 was considered above 1000 mg/kg bw for both male and female rats. Data are summarised in the table below.

Table 9: summary of mortality data in mice and rats (NTP, 1982)

Doses (mg/kg bw)	Mice		Rats	
	Male	Female	Male	Female
0	0/5	0/5	0/5	0/5
10	0/5	0/5	1/5	0/5
50	0/5	1/5	0/5	0/5
100	0/5	0/5	0/5	0/5
500	5/5	3/5	0/5	1/5
1000	5/5	5/5	2/5	0/5

In order to facilitate the decision for classification, and based on the data of the study, LD50 for mice are calculated by the dossier submitter. In accordance with the OECD guideline 425, the LD50 could be calculated using the maximum likelihood method. The LD50 then calculated would be **365 mg/kg bw** for females. No LD50 can be calculated for males as no mortality was found at 100 mg/kg bw and 100% mortality at the higher dose of 500 mg/kg bw.

Other available studies are of less quality.

Ban *et al.* (1995) administered a single dose of 200 mg/kg bw of vinylidene chloride in corn oil by gavage to male Swiss OF, mice. The purpose of the experiment was not the assessment of acute toxicity but the search of mechanism of nephrotoxicity and the design was as follow: groups of 10 mice received a single-dose of vinylidene chloride in association with various pre-treatments: group 1 = pre-treatment + vinylidene chloride; group 2 = alkaline solution/saline solution/distilled water + vinylidene chloride; group 3 = pre-treatment + corn oil; group 4 = alkaline solution/saline solution/distilled water + corn oil. In the purpose to classify VDC for its acute toxicity, only group 2 can be relevant. The observation period following the administration was 8 hours. No mortality was observed at the end of the 8 hours observation period, so the

LD50 value cannot be calculated but was considered **> 200 mg/kg bw in mice**. It has to be noted that this study is rated Klimish 1 by the DS in this report as it is considered a good quality study with regard to the objective of the authors. However, the aim was not to study the acute toxicity of vinylidene chloride or to derive a LD50, and as a consequence, it is of less relevance for classification purpose. Moreover, only one dose was tested which preclude the estimation of a LD50.

Jones *et al.* (1978a) administered single intragastric doses of vinylidene chloride in corn oil solution, over a range of 5 different concentrations to Alderley Park strain mice (6 mice/sex/group). LD50 values were calculated respectively for male and female animals from Thompson's (1947) method of moving averages and interpolation. No information on the control group, on the tested dose levels or on the post exposure observation period was given. The LD50 values obtained were **217 mg/kg bw for males mice** and **194 mg/kg bw for females mice** with no other information (including mortality).

The publication of Kennedy *et al.* (1991) is a survey of 108 chemicals conducted to determine the relationship between acute oral and acute inhalation toxicity data in the rat. As a consequence, no detail is available concerning the protocol used. The LD50 value reported by the authors is **2500 mg/kg bw in rats**.

Ponomarkov *et al.* (1980) administered vinylidene chloride in olive oil by gavage to groups of 4 inbred BDIV rats. The tested dose levels were not specified. No data on mortality was given in the publication. The LD50 value, calculated following the method described by Weil, was **1 800 mg/kg bw for males** and **1 500 mg/kg bw for females in rats**. In absence of sufficient information on study conditions and on results, the reliability of this data is considered limited.

Jenkins *et al.* (1972) administered vinylidene chloride to Holtzman male rats (number of animals/group not provided) by gavage in corn oil solution in a total volume of 2 mL/kg. Dose levels were not specified. Post exposure period of observation was 4 days. The LD50 value, calculated by the method of Litchfield and Wilcoxon (1949) was **1510 mg/kg bw in rats**.

10.1.1.2 Comparison with the CLP criteria

Exposure Route	Category 1	Category 2	Category 3	Category 4
Oral route (mg/kg bw)	ATE ≤ 5	5 < ATE ≤ 50	50 < ATE ≤ 300	300 < ATE ≤ 2000

The studies available lead to a wide range of LD50 (when able to be derived) depending on the species, leading to different categories of classification, from category 3 to no classification. Also, these studies are rather old and of various qualities (often with lack of details allowing proper interpretation), with none following current acute toxicity guidelines.

According to the ECHA CLP guidance (ECHA, 2017), "*Studies not considered suitable on reliability or other grounds should not be used for classification*" leaving only the NTP (1982) studies on rats and mice usable in this case. The LD50 of these studies are 50 mg/kg bw < LD50 < 500 mg/kg bw for mice, and > 1000 mg/kg bw for rats. In these studies, mice are therefore more sensitive than rats to the acute effects of vinylidene chloride, which seems confirmed by other acute studies. These observations can be considered robust, as studies by oral route with longer exposure (subacute, suchronic or chronic) also confirm this point (NTP, 1982) and the same conclusions are made from inhalation studies (NTP, 2015). This is further point out by toxicokinetics data. According to ECHA guidance (2017), "*In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. However, expert judgement may allow another ATE value to be used in preference, provided this can be supported by a robust justification*". As a consequence, the results of the NTP study on mice should be used for concluding on classification of VDC for acute toxicity. No LD50 was calculated by the NTP, but according to the data, it should be between 50 mg/kg bw (1/10 animals died) and 500 mg/kg bw (8/10 animals died). According to the maximum likelihood method, the LD50 is estimated to be 365 mg/kg bw for females. No LD50 can be calculated for males as no mortality was found at 100 mg/kg bw and 100% mortality at the higher dose of 500 mg/kg bw. This would lead, based on a precautionary principle considering 1) the heterogeneity of results, 2) the date of the key study, 3) the fact that no LD50 was derived, and 4) the overall robustness of the database, to a classification in category 3 (ATE > 50 mg/kg bw but ≤ 300 mg/kg bw). This proposal is also supported by the results from the Jones *et al.* (1978a) study which concluded to a LD50 of 217 mg/kg bw for male mice and 194 mg/kg bw for female mice (without further information).

Concerning the choice of the acute toxicity estimate (ATE) to calculate the acute toxicity of mixtures, the NTP study used for the classification did not derive a LD50 and therefore cannot be directly used to define an ATE. Considering the results of this study and the overall database, the converted acute toxicity point estimate (cATpE) for category 3, that is to said 100 mg/kg bw, appears not realistic as considerably too low. Therefore, based on a weight of evidence, it is proposed to use the study of Jones *et al.* (1978a) on mice, deriving LD50 of 194 and 217 mg/kg bw for females and males respectively. Consequently, a value of 200 mg/kg bw for the ATE based on this study seems to be the best way forward.

10.1.1.3 Conclusion on classification and labelling for acute oral toxicity

Regarding the data available, a classification as **Acute Tox. 3 H301: Toxic if swallowed** is warranted with an **ATE of 200 mg/kg bw**.

10.1.2 Acute toxicity - dermal route

Not assessed in this dossier.

10.1.3 Acute toxicity - inhalation route

Table 10: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** LC_{50}	**Reference**
Whole body vapour 4h exposure 14 days observation period	Sprague-Dawley rat 10/sex/dose	Vinylidene chloride Purity : 99.7%	7.94, 19.84, 35.71, 59.52 mg/L	LC_{50} for male and female: **28.35 and 40.78 mg/L** respectively. No classification	Anonymous, 1979a Klimisch 2
Whole body vapour 4h exposure 14 days observation period	Sprague-Dawley rat, fasted 10/sex/dose	Vinylidene chloride Purity : 99.7%	0.4 1, 2, 4, 6, 8, 20, 40, 48, mg/L	LC_{50} for fasted male and female: **1.63 and 26 mg/L** respectively. Acute Tox. 2 or No classification	Anonymous, 1979b Klimisch 2
Whole body vapour 4h exposure 14 days observation period	Male Sprague-Dawley rat 16/dose	Vinylidene chloride Purity not provided	4900, 6150 ppm Corresponding to 19.6, 24.6 mg/L	LC_{50} = **25.4 mg/L** No classification	Siegel, 1971 Klimisch 3
Whole body vapour 4h exposure 24h observation period	Male Holtzman rats, fasted or fed 5-10/dose	Vinylidene chloride Purity not provided	0.2 to 80 mg/L	Estimated LC50: Fed rats = **60 mg/L** Fasted rats = **2.4 ml/L** Acute Tox. 3 or no classification	Jaeger, 1974 Klimisch 3
Whole body vapour 22-23 h/d; 2-day exposure	CD rat 10/sex/dose	Vinylidene chloride Purity : 99%	0.060, 0.12 and 0.24 mg/L	no LD_{50}, no mortality	Short, 1977a Klimisch 3
Whole body vapour 22-23 h/d; 2-day exposure	CD-1 mouse 10/sex/dose	Vinylidene chloride Purity : 99%	0.060, 0.12 and 0.24 mg/L	LC_{50} (male, 22-23 h) = **0.39 mg/L (extrapolated to a 4 hours exposure using Haber's law: 2.34 mg/L)** LC_{50} (female, 22-23 h) = **0.42 mg/L (extrapolated to a 4 hours exposure using Haber's law: 2.52 mg/L)** Acute Tox. 3	Short, 1977a Klimisch 3
Whole body vapour 4h exposure 14 days observation period	NMRI mouse, fasted 10/sex/dose	Vinylidene chloride Purity : 99.7%	0.04, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/L	LC50 for fasted male and female**: 0.2 and 0.5 mg/L** respectively. Acute Tox. 1	Anonymous, 1979c Klimisch 2
Whole body vapour	NMRI mouse	Vinylidene chloride No information	No information	LC50 for male and female**: 0.46 and 0.82 mg/L** respectively.	Anonymous, 1979d

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** **LC_{50}**	**Reference**
4h exposure 14 days observation period		on purity		Acute Tox. 1 or 2	Klimisch 4[1]
Whole body vapour 4h exposure 14 days observation period	Chinese hamster, fasted 10-20/sex/dose	Vinylidene chloride Purity : 99.7%	0.5, 0.8, 1, 2, 3, 4, 6, 8 mg/L	LC_{50} for fasted male and female: **0.6 and 1.8 mg/L** respectively. Acute Tox. 2	Anonymous, 1979e Klimisch 2
Whole body vapour 4h exposure 14 days observation period	Chinese hamster 10/sex/dose	Vinylidene chloride Purity : 99.7%	1, 2, 3, 4, 6, 8, 12, 16, 20 mg/L	LC_{50} for male and female: **6.59 and 11.69 mg/L** respectively. Acute Tox. 3 or 4	Anonymous, 1979f Klimisch 2

[1] Unlike other studies from the same team (Anonymous a, b, c), this study was scored in Klimish 4 because the report was not available to the DS (as the others), but the study was also not summarised on ECHA disseminated website. Results were available from other secondary bibliographic sources. However, it can be reasonably expected that the protocol is similar to the others.

10.1.4 Short summary and overall relevance of the provided information on acute inhalation toxicity

All the studies investigating acute toxicity of VDC via inhalation route date from the 70's, before the implementation of technical guidelines for toxicological studies.

A series of studies aiming at comparing the sensitivity of 3 species to VDC (rats, mice and hamsters) and the effect of diet on the toxicity of the substance is of interest (Anonymous, 1979a; 1979b; 1979c; 1979d; 1979e; 1979f). In these studies, 10 animals/sex/concentration were whole body exposed to VDC for 4 hours, with a post exposure observation period of 14 days. From these studies, several conclusions can be drawn:

- effects of VDC are exacerbated in fasted animals ;
- regardless the species, males are consistently more sensitive to VDC than females;
- mice are more sensitive than hamsters, which are themselves more sensitive than rats.

Estimated LC50 in rats are rather high, except in fasted males (male and female: **28.35** and **40.78 mg/L**; fasted male and female: **1.63** and **26 mg/L**). Hamsters are more sensitive to effects of VDC, with estimated LC50 varying according the sex and diet status (fasted male and female: **0.6** and **1.8 mg/L**; male and female: **6.59** and **11.69 mg/L**). Finally, as observed through the oral route, mouse is the most sensitive species, with lower estimated LC50s (fasted male and female: **0.2** and **0.5 mg/L**; male and female: **0.46** and **0.82 mg/L**). It has to be noted that the LC50 presented here are the values presented by authors of the studies without details on the method of calculation used. However, regarding the data of mortality for some studies, when

available, some questions may arise, particularly in some cases when the number of dead animals drop from none to all between two tested concentrations (particularly, in the Anonymous (1979c) study, there were 3/10 male died at 0.2 mg/L and 10/10 at 0.3 mg/L ; 0/10 females died at 0.4 mg/L and 10/10 at 0.5 mg/L).

Other studies available are of less quality.

The study of Jaeger *et al.* (1974) lacks several informations as the precise concentrations used (even if the range is known), but the protocol seems similar to the recommended protocol for acute toxicity (5-10 male rats/dose whole body exposed to vapour 4 hours with a 24 hours observation period). Results (estimated LC50: Fed rats = **60 mg/L;** Fasted rats = **2.4 mg/L**) are concordant with the studies of Anonymous (1979a, b) in rats.

Short *et al.* (1977) exposed animals to VDC for 2 days, which is not in line with OECD guideline for acute toxicity.

In their study on male rats, Siegel *et al.* (1971) used only two concentrations and the LC50 derived is higher than the tested concentrations range (19.6 and 24.6 mg/L, LC50 = 25.4 mg/L).

10.1.5 Comparison with the CLP criteria

Exposure Route	Category 1	Category 2	Category 3	Category 4
Vapours (mg/L)	ATE ≤ 0.5	0.5 < ATE ≤ 2.0	2.0 < ATE ≤ 10.0	10.0 < ATE ≤ 20.0

Similarly to studies by oral route, the studies available for acute inhalation exposure lead to a wide range of LC50, leading to different categories of classification, from category 1 to no classification. Also, these studies are rather old and of various quality, with none following current acute toxicity guidelines.

Regarding the results, and particularly the series on mice, rats, and hamsters (Anonymous, 1979a, b, c, d; e, f), mice seem to be the most sensitive species to the acute effects of vinylidene chloride. This observation is confirmed by the overall database on the substance (subacute, subchronic or chronic exposure, oral or inhalation route (NTP, 1982; NTP, 2015)) and toxicokinetics data showing a higher body burden in mice than in rats. According to ECHA guidance (2017), "*In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. However, expert judgement may allow another ATE value to be used in preference, provided this can be supported by a robust justification*". As a consequence, the results of the studies on mice have to be used. The two studies of Anonymous (1979c, d) on fed and fasted mice lead to a classification in category 1, except for fed females for which the value lead to a category 2, based on the LC50 derived by authors (fasted male and female: 0.2

and 0.5 mg/L, fed male and female: 0.46 and 0.82 mg/L respectively). As the males seem consistently more sensitive than females after an acute inhalation exposure, a classification in category 1 should be warranted. As explained above, there are some uncertainties on the estimated LC50 values regarding the data on mortality reported, when available, and the lack of details provided by authors. Moreover, for the study on fed mice, which is the most relevant here for classification purpose, the mortality data are not available to allow a recalculation of LC50 values. DS is however of the opinion that these limitations do not call into questions the conclusion on classification in category 1 regarding the overall database.

Concerning the choice of the acute toxicity estimate (ATE) to calculate the acute toxicity of mixtures, DS would have proposed in a first place to use the converted acute toxicity point estimate (cATpE) for category 1 considering the above and the uncertainty on estimated LC50. However, the value of the cATpE, that is to say 0.05 mg/L, appears not realistic as considerably too low regarding the results of the two mice studies. In these two studies, the LC50 estimated by authors are lower in fasted animals: fasted male and female: 0.2 and 0.5 mg/L; fed male and female: 0.46 and 0.82 mg/L. However, it is known that this condition disturbs VDC metabolism, exacerbating toxicity of the compound. Also, fasting of animals is not requested in available technical guidances. Therefore, based on expert judgment and a weight of evidence regarding the results on mice, the value of **0.5 mg/L** for the ATE seems representative of the acute toxicity of VDC by inhalation.

10.1.6 Conclusion on classification and labelling for acute inhalation toxicity

Regarding the data available, a classification as **Acute Tox. 1 H330: Fatal if inhaled** is warranted with an **ATE of 0.5 mg/L**.

RAC evaluation of acute toxicity (oral and inhalation)

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The DS evaluated all six studies available for acute oral toxicity and in a weight of evidence approach proposed classification as Acute Tox. 3; H301: Toxic if swallowed with an acute toxicity estimate (ATE) of 200 mg/kg bw. The DS reached the conclusion based on two studies, the NTP (1982) (Klimisch 1) as the main study for classification and the Jones *et al.* (1978a) (Klimisch 4) as a supportive study also used for the determination of the ATE and by using the most sensitive species, the mouse.

No LD_{50} was calculated by the NTP, but according to the data, it should be between 50 mg/kg bw (1/10 animals died) and 500 mg/kg bw (8/10 animals died). The LD_{50} was estimated to be 365 mg/kg bw for females (maximum likelihood method). No LD_{50} could be calculated for males as no mortality was found at 100 mg/kg bw and 100% mortality occurred at the higher dose of 500 mg/kg bw. Although the LD_{50} from the NTP study was

considered to warrant classification in Category 4, the DS argued that based on a precautionary principle and considering 1) the heterogeneity of results, 2) the date of the key study, 3) the fact that no LD_{50} was derived, and 4) the overall robustness of the database, a classification in Category 3 (50 mg/kg bw < ATE ≤ 300 mg/kg bw) was justified. This proposal was also supported by the results from the Jones *et al.* (1978a) study, which concluded an LD_{50} of 217 mg/kg bw for male mice and 194 mg/kg bw for female mice (without further information). The proposed LD_{50} of 200 mg/kg bw was rounded from the results of the Jones *et al.* (1978a) study.

Acute inhalation toxicity

The DS evaluated nine available studies and proposed classification as Acute Tox. 1; H330: Toxic if inhaled with an ATE of 0.5 mg/L based on the most sensitive species, the male mouse (Anonymous, 1979d).

In order to compare the sensitivity of 3 species to VDC (rats, mice and hamsters) and the effect of diet on the toxicity of the substance, the DS relied on Anonymous (1979a; 1979b; 1979c; 1979d; 1979e; 1979f). LC_{50} values were derived in two studies (Anonymous, 1979c, d) on fed and fasted mice: fasted males and females: 0.2 and 0.5 mg/L, respectively; fed males and females: 0.46 and 0.82 mg/L, respectively. Males were found consistently more sensitive than females after an acute inhalation exposure and therefore, classification in Category 1 was proposed by the DS with an ATE rounded to 0.5 mg/L based on the LC_{50} of fed male mice.

Comments received during consultation

Acute oral toxicity

There were two comments submitted during the consultation, one by a Member State Competent Authority (MSCA) and one by an Industry/Trade Association.

The MSCA questioned the proposed category of classification, suggesting Category 4 based on the LD_{50} value of 365 mg/kg bw (female mice) derived from the most reliable NTP study (1982). In addition, even in the case of a Category 3 classification, the MSCA argued that the converted acute toxicity point estimate (cATpE) of 100 mg/kg bw was unrealistic, and an ATE of 200 mg/kg bw could be proposed based on the worst-case scenario of the Jones *et al*. (1978a) study.

The DS replied that the NTP study, despite its high quality did not meet the current guidelines as it was not conducted to determine an LD_{50}. The value of 365 mg/kg bw, which was close enough to the upper ATE limit of Category 3, corresponded to female mice, which were expected to be less sensitive than male mice, based on the toxicological profile of VDC. Therefore, a lower LD_{50} was expected, pointing to a Category 3 classification, in line with the results of the other less reliable (lower Klimisch score) available studies.

In the second comment, Industry/Trade Association proposed that based on metabolic differences between different species, the toxicity observed in rats was the most representative of the toxicity expected in humans, and the acute classification should be based on the LD_{50} from rat studies. The lowest LD_{50} value observed in rats was 1500 mg/kg bw in female rats (Ponomarkov *et al*. 1980). Based on this value, Industry/Trade Association considered that VDC should be classified in Category 4; H302: Harmful if swallowed.

The DS responded that, in the absence of robust data, the Guidance on the Application of

the CLP Criteria (CLP guidance) recommends using the most sensitive species, which is the reason why data on mice were used.

Acute inhalation toxicity

There were two comments regarding acute inhalation toxicity, one from a MSCA and one from Industry/Trade Association.

The MSCA supported the proposed classification as Acute Tox. 1; H330 with an ATE of 0.5 mg/L.

Similarly to the comment on acute oral toxicity, the Industry/Trade Association proposed that based on the metabolism differences between the species, the toxicity observed in rats was the most representative of the toxicity expected in humans, and the acute inhalation classification should be based on the LC_{50} from rat studies. For acute inhalation toxicity, 6 studies conducted in rats were available. The lowest LC_{50} reported for rats was 28.350 mg/L/4 h (Anonymous, 1979a), which did not warrant classification. However, there was a harmonised classification for VDC in Annex VI of CLP as Acute Tox. 4; H332. The Industry/Trade Association proposed to maintain this classification as a conservative approach.

The DS responded in the same manner as in the acute oral toxicity section.

Assessment and comparison with the classification criteria

Acute oral toxicity

Table: *Summary of acute oral toxicity studies*

Method, guideline, duration of exposure deviations if any	Species, strain, sex, no/group	Test substance	Dose levels	Value LD_{50} Classification	Reference
Gavage Vehicle: corn oil Observation period: 14 days	Mice B6C3F1, male and female 5 animals/sex/dose	Vinylidene chloride Purity: 99 %	0, 10, 50, 100, 500 and 1000 mg/kg bw	50 mg/kg bw < LD_{50} < 500 mg/kg bw **Acute Tox. 3 or 4**	NTP, 1982 Klimisch 1 Key study
	Rat Fischer 344, male and female 5 animals/sex/dose			LD_{50} > 1000 mg/kg bw **Acute Tox. 4 or no classification**	
Gavage	Swiss OF, mice (IFFA-CREDO), Males (10/group)	Vinylidene chloride	200 mg/kg bw	LD_{50} > 200 mg/kg bw **Acute Tox. 3 or 4 or no classification**	Ban *et al*., 1995
Gavage Vehicle: corn oil	Holtzman male rats Number of	Vinylidene chloride Purity not	Not specified	LD_{50} = 1510 mg/kg bw **Acute Tox. 4**	Jenkins *et al*., 1972 Klimisch 3

	animals/group not provided	provided			
Gavage Vehicle: olive oil	Inbred BDIV rats 4 animals/sex/dose	Vinylidene chloride Purity: 99 %	Not specified	LD_{50} = 1800 mg/kg bw for males, 1500 mg/kg bw for females. **Acute Tox. 4**	Ponomarkov *et al*., 1980 Klimisch 3
Intragastric Vehicle: corn oil	Alderley Park male and female mice 6 animals/sex/dose	Vinylidene chloride Purity not provided	5 groups, doses not specified	LD_{50} male = 217 mg/kg bw LD50 female = 194 mg/kg bw **Acute Tox. 3**	Jones *et al*., 1978a Klimisch 4
Oral, not further specified	Rat, strain and sex not specified	Vinylidene chloride Purity not provided	Not specified	LD_{50} = 2500 mg/kg bw **No classification**	Kennedy *et al*., 1991 Klimisch 4

The six available studies for acute oral toxicity are shown in the table above. Although most of these studies are old, not conducted according to current guidelines and lack proper reporting, they are published in well-respected peer-reviewed journals.

It is apparent from the table above that the most sensitive species is the mouse, with LD_{50} values ranging from 194 to 365 mg/kg bw for the most sensitive sex in each study. For rats, the respective LD_{50} values are > 1000 mg/kg bw. Classification should be based on mice because according to the CLP guidance (version 5, 2017): "*In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. However, expert judgement may allow another ATE value to be used in preference, provided this can be supported by a robust justification*".

The most reliable study is the NTP (1982) study which, however, was a single range-finding study conducted as a preliminary assessment of toxicity aiming at studying the carcinogenic potency of VDC. Therefore, the authors did not derive an LD_{50}. The study was conducted both in mice and rats. The conditions of the study are listed in the table above and the mortalities observed in the table below.

Table: *Mortality data in mice and rats (NTP, 1982)*

Dose mg/kg bw	**Mice** #mortalities/#animals		**Rats** #mortalities/#animals	
	Male	Female	Male	Female
0	0/5	0/5	0/5	0/5
10	0/5	0/5	1/5	0/5
50	0/5	1/5	0/5	0/5
100	0/5	0/5	0/5	0/5
500	5/5	3/5	0/5	1/5
1000	5/5	5/5	2/5	0/5

For mice, the LD_{50} lies between 50 mg/kg bw (males: no lethality, females: 20 % lethality) and 500 mg/kg bw (males: 100% lethality, females: 60% lethality).

In accordance with the OECD TG 425, an LD_{50} of 365 mg/kg bw was estimated by the DS (Acute Tox. 4) using the maximum likelihood method for female mice. No LD_{50} can be

calculated for males as no mortality was seen at 100 mg/kg bw and 100% mortality was observed at 500 mg/kg bw.

In the Ban *et al.* (1995) study, an LD_{50} could not be derived, but it is expected to be > 200 mg/kg bw, since no mortalities were observed in mice at 200 mg/kg bw with a monitoring period of 8 hours. It should be also noted that the study was not conducted as an acute oral toxicity study but rather to examine the mechanism of nephrotoxicity.

In the Jones *et al*. (1978a) study, published in the Br. J. Cancer, groups of mice were given a single oral dose of the test substance by gavage in corn oil at 5 dose levels. The LD_{50} values were calculated by using the Thompson's (1947) method of moving averages and interpolation both for male and female mice. No information on the control group, on the tested dose levels or on the post exposure was provided. At the time of this study, no guideline was available either for the method used or for the GLP. The LD_{50} values reported were 217 mg/kg bw for male mice and 194 mg/kg bw (within guidance value range for Acute Tox. 3) for female mice with no other information (including data on mortality rates).

In addition to the Jones *et al*. (1978a) study, in the micronucleus test in mice by Sawada *et al*. (1987), although not conducted for acute oral toxicity purposes, a 50 % mortality in male mice was observed at a single dose of 200 mg/kg bw, which could further support the proposed ATE.

The results of the available studies point to classification as acute oral toxicity, either Category 3 or Category 4.

RAC agrees with the DS that the results of the NTP study on mice should be used for classification purposes, with the Jones *et al*. (1978a) study as supporting evidence. The value of LD_{50} 365 mg/kg bw for female mice, as estimated by the DS using the maximum likelihood method, warrants classification in Category 4, but is close to the threshold value of ATE 300 mg/kg bw for Category 3 (22% higher). The Jones *et al*. (1978a) study supports Category 3 with an LD_{50}=194 mg/kg bw for female mice. These findings would lead to a borderline case between Category 3 (Jones *et al.,* 1978a) and 4 (NTP, 1982). In a weight of evidence approach, also taking into consideration the 50% mortality at 200 mg/kg bw observed in the Sawada *et al*. (1987) micronucleus study, RAC considers that classification as Acute Tox. 3 is justified.

RAC finds the cATpE for Category 3 (100 mg/kg bw), not to be realistic and the ATE of 200 mg/kg bw proposed by the DS too conservative, as explained above. Therefore, the upper ATE limit of Category 3, ATE of 300 mg/kg bw, is proposed.

In conclusion, RAC considers that classification of VDC as **Acute Tox. 3; H301: Toxic if swallowed** with an **ATE of 300 mg/kg bw** is warranted.

Acute inhalation toxicity

Table: *Summary of acute inhalation toxicity studies*

Method, guideline, duration of exposure deviations if any	Species, strain, no/group	Test substance, form and particle size (MMAD)	Dose levels	LC_{50}	Reference
Whole body vapour	Sprague-Dawley rat	Vinylidene chloride	7.94, 19.84, 35.71, 59.52	LC_{50} for males and females:	Anonymous, 1979a

4-h exposure 14-day observation period	10/sex/dose	Purity: 99.7%	mg/L	**28.35 and 40.78 mg/L,** respectively. No classification	Klimisch 2
Whole body vapour 4-h exposure 14-day observation period	Sprague-Dawley rat, **fasted** 10/sex/dose	Vinylidene chloride Purity: 99.7%	0.4, 1, 2, 4, 6, 8, 20, 40, 48, mg/L	LC_{50} for fasted males and females: **1.63 and 26 mg/L,** respectively. Acute Tox. 2 or No classification	Anonymous, 1979b Klimisch 2
Whole body vapour 4-h exposure 14-day observation period	Male Sprague-Dawley rat 16/dose	Vinylidene chloride Purity not provided	4900, 6150 ppm Corresponding to 19.6, 24.6 mg/L	LC_{50} = **25.4 mg/L** No classification	Siegel, 1971 Klimisch 3
Whole body vapour 4-h exposure 24-h observation period	Male Holtzman rats, fasted or fed 5-10/dose	Vinylidene chloride Purity not provided	0.2 to 80 mg/L	Estimated LC_{50}: Fed rats = **60 mg/L** Fasted rats = **2.4 mg/L** Acute Tox. 3 or no classification	Jaeger, 1974 Klimisch 3
Whole body vapour 22-23 h/d; 3-day exposure	CD rat 10/sex/dose	Vinylidene chloride Purity: 99%	0.060, 0.12 and 0.24 mg/L	No LD_{50}, no mortality	Short, 1977a Klimisch 3
Whole body vapour 22-23 h/d; 1-day exposure	CD-1 mouse 10/sex/dose	Vinylidene chloride Purity: 99%	0.060, 0.12 and 0.24 mg/L	LC_{50} (males, 22-23 h) = **0.39 mg/L (extrapolated to a 4-h exposure using Haber's law: 2.34 mg/L)** LC_{50} (females, 22-23 h) = **0.42 mg/L (extrapolated to a 4-h exposure using Haber's law: 2.52 mg/L)** Acute Tox. 3	Short, 1977a Klimisch 3
Whole body vapour	NMRI mouse,	Vinylidene chloride	0.04, 0.08, 0.1, 0.2, 0.3,	LC_{50} for fasted males and	Anonymous, 1979c

4-h exposure 14-day observation period	fasted 10/sex/dose	Purity: 99.7%	0.4, 0.5, 0.6 mg/L	females**: 0.2 and 0.5 mg/L,** respectively. Acute Tox. 1	Klimisch 2
Whole body vapour 4-h exposure 14-day observation period	NMRI mouse	Vinylidene chloride No information on purity	No information	LC_{50} for males and females**: 0.46 and 0.82 mg/L,** respectively. Acute Tox. 1 or 2	Anonymous, 1979d Klimisch 4
Whole body vapour 4-h exposure 14-day observation period	Chinese hamster, fasted 10-20/sex/dose	Vinylidene chloride Purity: 99.7%	0.5, 0.8, 1, 2, 3, 4, 6, 8 mg/L	LC_{50} for fasted males and females: **0.6 and 1.8 mg/L,** respectively. Acute Tox. 2	Anonymous, 1979e Klimisch 2
Whole body vapour 4-h exposure 14-day observation period	Chinese hamster 10/sex/dose	Vinylidene chloride Purity: 99.7%	1, 2, 3, 4, 6, 8, 12, 16, 20 mg/L	LC_{50} for males and females: 6.59 and 11.69 mg/L, respectively. Acute Tox. 3 or 4	Anonymous, 1979f Klimisch 2

All ten studies evaluated in the CLH report have with deficiencies and were not performed according to OECD test guidelines. However, there is a series of studies by Anonymous (1979a-1979f), which aimed to assess acute inhalation toxicity of VDC in rats, hamsters and mice and to evaluate the effect of the animals' nutritional status (fasting) on inhalation toxicity. These studies were conducted according to a protocol similar to the current guidelines and they were considered as more reliable by the DS. RAC, in agreement with the DS, bases the classification for acute inhalation toxicity on these studies by Anonymous.

In these studies, 10 animals/sex/dose were whole-body exposed to VDC for 4 hours, with a post-exposure observation period of 14 days. It is noted that there are reporting deficiencies.

A comparison of the estimated LC_{50} values from the Anonymous (1979a-1979f) studies is summarised in the table below.

Table: *Comparison of LC_{50} values from the Anonymous (1979a-1979f) studies*

Method, guideline, duration of exposure	**Species, strain, nutritional status, no/group**	**Dose levels**	**LC_{50} Male**	**LC_{50} Female**	**Reference**
Whole body vapour 4-h exposure	Sprague-Dawley rat **non-fasted** 10/sex/dose	7.94, 19.84, 35.71, 59.52 mg/L	**28.35 mg/L** No classification	**40.78 mg/L** No classification	Anonymous, 1979a Klimisch 2

14-day observation period	Sprague-Dawley rat, **fasted** 10/sex/dose	0.4, 1, 2, 4, 6, 8, 20, 40, 48, mg/L	**1.63 mg/L** Acute Tox. 2	**26 mg/L** No classification	Anonymous, 1979b Klimisch 2
Whole body vapour 4-h exposure 14-day observation period	Chinese hamster **non-fasted** 10/sex/dose	1, 2, 3, 4, 6, 8, 12, 16, 20 mg/L	**6.59 mg/L** Acute Tox. 3	**11.69 mg/L** Acute Tox. 4	Anonymous, 1979f Klimisch 2
	Chinese hamster, **fasted** 10-20/sex/dose	0.5, 0.8, 1, 2, 3, 4, 6, 8 mg/L	**0.6 mg/L** Acute Tox. 2	**1.8 mg/L** Acute Tox. 2	Anonymous, 1979e Klimisch 2
Whole body vapour 4-h exposure 14-day observation period	NMRI mouse **non-fasted** 10/sex/dose	No information	**0.46 mg/L** Acute Tox. 1	**0.82 mg/L** Acute Tox. 2	Anonymous, 1979d Klimisch 4 (due to reporting deficiencies)
	NMRI mouse, **fasted** 10/sex/dose	0.04, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/L	**0.2 mg/L** Acute Tox. 1	**0.5 mg/L** Acute Tox. 1	Anonymous, 1979c Klimisch 2

The following conclusions can be drawn from the data shown in the table above:

- mice are more sensitive than hamsters, which are more sensitive than rats;
- regardless of the species, males are consistently more sensitive to VDC than females;
- effects of VDC are exacerbated in fasted animals

It should be noted that the LC_{50} values discussed are those provided by the authors of the studies without any details on the calculation method used. Moreover, for the Anonymous (1979d) study, the data on mortalities per dose are not available. In addition, in the Anonymous (1979c) study, mortality reporting between consecutive doses seems unusual: 3/10 male died at 0.2 mg/L and 10/10 at 0.3 mg/L; 0/10 females died at 0.4 mg/L and 10/10 at 0.5 mg/L).

In rats the LC_{50} ranged from 1.63 mg/L (for fasted male rats) to 40.78 mg/L (for non-fasted female rats). Hamsters were more sensitive to the toxicity of VDC, with estimated LC_{50} varying according to sex and diet status from 0.6 mg/L (for fasted male hamsters) to 11.59 mg/L (for non-fasted females).

Lastly, in accordance with the results from the oral studies, the mouse proved to be the most sensitive species, with estimated LC_{50} values ranging from 0.2 mg/L for fasted males to 0.82 mg/L for non-fasted females.

Regarding classification, according to the CLP guidance (version 5, 2017) *"In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. However, expert judgement may allow another ATE value to be used in preference, provided this can be supported by a robust justification".* Consequently, classification should be based on the results of the mouse studies, as the

most sensitive species and the findings of the Anonymous (1979a-f) (Klimisch 2) studies, as the most reliable ones.

Since the nutritional status of fasting is known to affect the metabolism of VDC negatively and it is also not a condition/parameter of current testing guidelines, RAC agrees with the DS to use data on non-fasted male mice (Anonymous, 1979d). All relevant studies in male mice lead to a classification for acute inhalation toxicity, Category 1. Concerning the acute toxicity estimate (ATE), RAC agrees with the DS that the value of the cATpE (0.05 mg/L) appears not realistic as it is considered too low in comparison to the results of the two Anonymous (1979c, d) mouse studies. Therefore, RAC supports the proposed rounded ATE of 0.5 mg/L based on the data from Anonymous (1979d).

In conclusion, RAC agrees with the proposal by the DS that classification of VDC as **Acute Tox. 1; H330: Toxic if inhaled with an LC_{50} of 0.5 mg/L (vapours**) is warranted.

10.2 Skin corrosion/irritation

Not assessed in this dossier.

10.3 Serious eye damage/eye irritation

Table 11: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
One eye of each rabbits treated with 50 µL of undiluted VDC solution and not washed. Same dose of physiological solution applied in the non treated eye as control. Post exposure time points: 1h, 24h and 8 days	Vienna white rabbits	vinylidene chloride Purity: 99.7%	Undiluted No washing	1h after treatment: slight redness and slight edema. 24h after application: slight redness. 1 week after treatment: no significant effect.	Anonymous, 1979g Klimisch 3

Table 12: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Guideline: yes OECD TG 437 (Bovine Corneal Opacity and Permeability test method or BCOP assay) GLP: yes	vinylidene chloride purity > 99%	VDC tested undiluted. Corneae opacity measured using the OP-KiT opacitometer. Permeability of the cornea possibly caused by the test item, measured at 490 nm (OD490) with a spectrophotometer.	Mean IVIS = 43.90.	Anonymous, 2010 Klimisch 1

10.3.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

An *in vivo* study was conducted on 2 Vienna white rabbits (Anonymous, 1979g). VDC was tested undiluted. One eye of each rabbit was treated with 50 µL and not washed. The same dose of physiological solution was applied in the non treated eye as control. The post exposure observation period was up to 8 days, with time points at 1h, 24h and 8 days after treatment. Individual detailed results were not available.

One hour after treatment, slight redness and slight edema was observed. Only slight redness was still found 24h after application. After one week, no significant effect was noted except a slight irritation of the lining of the eye. Considering the lack of details (only summary available) and deviations from current OECD TG guideline (e.g. observation period), the study was considered not reliable.

The irritation potential of VDC was assessed in an *in vitro* test on fresh bovine cornea, complying to the current GLP and OECD test guideline 437 (Bovine Corneal Opacity and Permeability test method or BCOP assay) requirements (Anonymous, 2010). This study is therefore considered fully reliable.

VDC was tested undiluted. Three corneae were used in each group (test item, negative control, positive control). The negative control was a 0.9% NaCl solution and the positive control, 2-Ethoxyethanol.

The corneae opacity was measured using the OP-KiT opacitometer. In the second step of the assay, permeability of the cornea, as possibly caused by the test item, was measured at 490 nm (OD490) with a spectrophotometer.

With the negative control, neither an increase of opacity nor permeability of the corneae could be observed. The positive control showed clear opacity and distinctive permeability of the corneae and is therefore considered as severe eye irritant. VDC caused only a slight increase of opacity values but a distinct increase of the permeability values of the corneae compared with the results of the negative control. The calculated mean in vitro score was 43.90. Results are detailed in the table below.

Table 13: Results of VDC tested in BCOP assay after 10 minutes incubation time

Test group	Opacity value *		Permeability OD490**		*In vitro* score	Mean *in vitro* score
Negative control	-1	Mean 0.00	0.052	Mean 0.055	-0.22	0.82
	0		0.051		0.77	
	1		0.061		1.92	
Positive control	66.00		0.640		75.61	79.20
	68.00		0.874		81.12	
	69.00		0.791		80.87	
VDC	10.00		2.312		44.69	43.90
	9.00		2.306		43.60	

	8.00		2.360		43.41	

*: Difference (t130-t0) of opacity / **: Optical density at 490 nm

On the basis of these results, the test item is not corrosive and could be considered as moderate eye irritant.

10.3.2 Comparison with the CLP criteria

According to the results of the BCOP assay (mean IVIS = 43.90), VDC cannot be considered as a serious eye damaging substance and therefore does not warrant a classification in category 1. The OECD Guideline (TG 437) for BCOP assay specifies that if the IVIS score is between 3 and 55, no stand-alone prediction can be made. However, this is the only reliable test on eye irritancy available with VDC.

Moreover, the guideline also indicates that : "*The BCOP test method is not recommended for the identification of test chemicals that should be classified as irritating to eyes (UN GHS Category 2 or Category 2A) or test chemicals that should be classified as mildly irritating to eyes (UN GHS Category 2B). […] A chemical that is not predicted as causing serious eye damage or as not classified for eye irritation/serious eye damage with the BCOP test method would require additional testing (in vitro and/or in vivo) to establish a definitive classification.*", which is in line with the ECHA CLP guidance (2017) ("*There are currently no validated in vitro eye irritation test methods available*").

Therefore, data available are not sufficient to draw a conclusion about classification, as an irritant potency of vinylidene chloride cannot be excluded. No classification can be proposed.

10.3.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Based on ECHA CLP guidance (2017), data available are inconclusive and not sufficient for classification. Consequently, no classification is proposed for VDC.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS evaluated the two available studies for the classification of serious eye damage/eye irritation.

The first study (Anonymous, 1979g), although of low reliability, showed slight redness and slight oedema but the effects were reversible after 1 week.

The second study was an *in vitro* test on fresh bovine cornea, complying with the current GLP and OECD test guideline 437 (Bovine Corneal Opacity and Permeability test method or BCOP assay) requirements (Anonymous, 2010). The calculated mean *in vitro* score, mean IVIS = 43.90 of the study does not warrant a classification in Category 1. The OECD TG 437 for BCOP assay specifies that if the IVIS score is between 3 and 55, no stand-alone prediction can be made. Since this was the only available reliable study, the DS proposed no classification for VDC for serious eye damage/eye irritation due to inconclusive data.

Comments received during consultation

There was one comment by a MSCA that based on the same reasoning as the DS, supported the proposed no classification, as no further *in vitro* tests were available, and "no classification due to insufficient data".

Assessment and comparison with the classification criteria

The data for the two available studies, *in vivo* and *in vitro* (BCOP), respectively, are shown in the table below.

***Table**: Summary table of available animal studies on serious eye damage/eye irritation*

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results - Observations and time point of onset - Mean scores/animal - Reversibility**	**Reference**
No standard method No guideline One eye of each rabbit treated with 50 µL of undiluted VDC solution and not washed. Same dose of physiological solution applied in the non-treated eye as control. Post exposure observation time points: 1h, 24h and 8 days	Vienna white rabbits 2 animals	vinylidene chloride Purity: 99.7%	50 µL undiluted No washing	1h after treatment: slight redness and slight edema. 24h after application: slight redness. 1 week after treatment: no significant effect.	Anonymous, 1979g Klimisch 3

***Table:** Summary table of available in vitro studies for serious eye damage/eye irritation*

Guideline Type of study	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**

Guideline: yes OECD TG 437 (Bovine Corneal Opacity and Permeability test method or BCOP assay) GLP: yes	vinylidene chloride purity > 99%	VDC tested undiluted. Cornea opacity measured using the OP-KiT opacitometer. Permeability of the cornea possibly caused by the test item, measured at 490 nm (OD490) with a spectrophotometer.	Mean IVIS = 43.90.	Anonymous, 2010 Klimisch 1

The *in vivo* study did not follow current test guidelines and was considered as Klimisch 3 by the DS. The study was conducted on 2 Vienna white rabbits with undiluted VDC and without washing of the eye. The same dose of physiological solution was applied in the non-treated eye as control. The post exposure observation period was up to 8 days, with observation time points at 1h, 24h and 8 days after treatment. Individual detailed results were not available. Nonetheless, an hour after exposure slight redness and slight edema were observed in both animals. After one week, no significant effect was noted except a slight irritation of the lining of the eye thus rendering the effects reversible.

The *in vitro* test was performed according to OECD TG 437 (BCOP - Bovine Corneal Opacity and Permeability test method or BCOP assay) and to GLP and therefore, was considered as Klimisch 1 by the DS.

The BCOP test method is an organotypic model that provides short-term maintenance of normal physiological and biochemical function of the bovine cornea *in vitro*. In this test method, damage by the test chemical is assessed by quantitative measurements of changes in corneal opacity and permeability with an opacitometer and a visible light spectrophotometer, respectively. The BCOP test method uses isolated corneas from the eyes of freshly slaughtered cattle (calf or bovine). Corneal opacity is measured quantitatively as the amount of light transmission through the cornea. Permeability is measured quantitatively as the amount of sodium fluorescein dye that passes across the full thickness of the cornea, as detected in the medium in the posterior chamber. The mean opacity and permeability values for each treatment group are combined in an empirically-derived formula to calculate an *in vitro* irritancy score (IVIS).

IVIS = mean opacity value (OP-KIT) + (15 x mean permeability OD490)

Based on the IVIS score of the BCOP test method, a chemical can be either classified as serious eye damage/eye irritation, Category 1 (IVIS > 55) or not classified at all for serious eye damage/eye irritation (IVIS ≤ 3). No conclusion for classification can be made based on IVIS score of the BCOP 3 < IVIS ≤ 55 and further testing using *in vivo* methods is required. According to the CLP guidance (2017), a substance can be considered as causing serious eye damage (Category 1) based on positive results in the BCOP test. However, according to the CLP guidance, there are no *in vitro* tests with regulatory acceptance for eye irritation at present.

In the Anonymous (2010) study, VDC was tested undiluted, and three corneas were used in

each group (test item, negative control and positive control). The negative control was a 0.9% NaCl solution and the positive control was 2-Ethoxyethanol.

The cornea opacity was measured using the OP-KiT opacitometer. In the second step of the assay, permeability of the cornea, as possibly caused by the test item, was measured at 490 nm (OD490) with a spectrophotometer. The results are shown in the Table below.

Table: *Results of VDC tested in BCOP assay after 10 minutes incubation time*

Test Group	Opacity Value		Permeability		In vitro score	IVIS
Negative Control	-1	Mean 0	0.052	Mean 0.055	-0.22	Mean 0.82
	0		0.051		0.77	
	1		0.061		1.92	
Positive Control	66.00	67.67	0.640	0.768	75.61	79.20
	68.00		0.874		81.12	
	69.00		0.791		80.87	
VDC	10.00	9.00	2.312	2.326	44.69	43.90
	9.00		2.306		43.60	
	8.00		2.360		43.41	

The calculated mean *in vitro* irritancy score (IVIS) for VDC is:

IVIS = 9 + 15 X 2.326 = 43.90

Based on this IVIS score, VDC cannot be considered as meeting the criteria for serious eye damage and therefore does not warrant classification in Category 1. No other stand-alone prediction can be made as explained above.

In conclusion, the results from Anonymous (1979g) study are not suitable for classification purposes and the results from the Anonymous (2010) study exclude classification for serious eye damage/(Category 1), but are not sufficient to draw a conclusion on no classification for eye irritation (Category 2), as the irritant potency of VDC cannot either be excluded or verified with the BCOP assay.

Therefore, RAC proposes **no classification of VDC for serious eye damage/eye irritation due to inconclusive data.**

10.4 Respiratory sensitisation

Not assessed in this dossier.

10.5 Skin sensitisation

Not assessed in this dossier.

10.6 Germ cell mutagenicity

Table 14: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Tests on bacteria				
Bacterial gene mutation Ames Test Deviations: 4 strains instead of 5 GLP: no information	Vinylidene Chloride Purity: 98%	S. typhimurium strains TA1535, TA1537, TA98 and TA100. With and without metabolic activation (from rat and hamster liver) Vehicle : DMSO Positive controls: sodium azide for TA1535 and TA 100,4-nitro-o-phenylenediamine or TA98, and 9-aminoacridine for TA97 and TA1537; 2-aminoanthracene was used with all strains with hamster and rat liver metabolic activation systems. Concentrations: 0, 33.3, 100, 333.3, 1000 and 3333.3 µg/plate (selected based on a preliminary dose setting experiment)	Negative (+/- S9 mix) Test conducted without desiccator and without any measures to prevent volatilisation	Mortelmans et al., 1986 Klimisch 2
Bacterial gene mutation Ames Test GLP: no information	Vinylidene Chloride Purity: 99.996%	S. typhimurium strains TA1535, TA1537, TA98, TA100 and TA92 E. coli WP2 With and without metabolic activation (S9 fractions of liver or kidney from mouse, hamster, rat or human) Concentrations : 375 ; 2250 ; 4500 ; 10500 ; 22500 ppm Negative control: yes Positive control: no information	+ S9 mix: Positive - S9 mix: Negative	Oesch et al., 1983 Klimisch 2
Bacterial gene mutation Ames Test Deviations: only one strain and one concentration tested GLP: no information	Vinylidene Chloride Purity not provided	E. coli K-12 With and without metabolic activation (from mice liver) Concentration: 2.5 mM No information on negative and positive controls, vehicle, number of replicates.	+ S9 mix: Positive - S9 mix: negative No cytotoxicity	Greim et al., 1975 Klimisch 3
Bacterial gene mutation Ames Test Deviations: only 2 strains and 3	Vinylidene Chloride Purity not provided	S. typhimurium strains TA1530 and TA100. 4h exposure With metabolic activation (mice and	Positive (+ S9 mix)	Bartsch et al., 1975 Klimisch 3

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
concentrations tested GLP: no information		rat liver, kidney and lung) Concentrations: 0.2, 2 or 20% in air No information on vehicle, positive control.		
Bacterial gene mutation Ames Test GLP: no information	Vinylidene Chloride Purity not provided	S. typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100. Without metabolic activation Vehicle : DMSO Positive control: 4-nitroquinoline-N-oxide. Information on negative controls, number of replicates, tested concentrations not provided.	Negative for TA100 (only result provided) No information about the presence of precipitate or toxicity available	Laumbach et al., 1977[a] Klimisch 4
Bacterial gene mutation Ames Test GLP: no information	Vinylidene Chloride (used as the positive control) Purity not provided	S. typhimurium TA100 With and without metabolic activation (no more detail) Concentration: 5% Vehicle : DMSO Information on negative controls, number of replicates not provided.	Positive with metabolic activation No information about the presence of precipitate or toxicity available	Simmon et al., 1977[a] Klimisch 4
Bacterial gene mutation Ames Test GLP: no information	Vinylidene Chloride (used as the positive control) Purity not provided	S. typhimurium strains TA98 and TA100 (only results with TA100 provided in the publication). With and without metabolic activation (from rat liver) Concentration: 5% Test in closed containers (aqueous phase was saturated with VDC). No information on negative controls and vehicle.	TA 100: Positive (+/- S9 mix) No information about the presence of precipitate or toxicity available	Waskell, 1978 Klimisch 4
Bacterial gene mutation Ames Test Deviations: only 2 strains GLP: no information	Vinylidene Chloride (used as positive control) Purity not provided	S. typhimurium, TA1535 and TA100 With metabolic activation (from rat, hamster and mice liver) Concentration: 3% No information on negative control.	Positive	Baden et al., 1978 Klimisch 4
Bacterial gene mutation Ames Test Deviations: only one concentration,	Vinylidene Chloride Purity not provided	S. typhimurium, TA1535 and TA100 With metabolic activation (rat, mouse, marmoset, human S9 fractions)	Weakly positive with kidney and liver S-9 mix from normal mice, but strongly positive with the S-9 mix from induced animals. In rat tissue,	Jones & Hathway, 1978b Klimisch 4

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
2 strains GLP: no information		Concentration: 5% in air Negative control: yes Positive control: no information	positive response only in induced animals.	
Bacterial gene mutation Ames Test Deviations: only 2 strains GLP: no information	Vinylidene Chloride (used as positive control) Purity not provided	S. typhimurium, TA1535 and TA100 With metabolic activation (from rat liver) Concentration: 3% Negative control: yes	Positive	Baden et al., 1982 Klimisch 4
Ara mutagenicity assay No OECD guideline GLP: no information	Vinylidene Chloride Purity: 99.5%	S. typhimurium strains BA13 (mutation indicator) and BAL13 (survival indicator) With and without metabolic activation (from rat liver) Vehicle : DMSO 5 tested concentrations (no more information) No information on controls.	Positive (+ S9 mix)	Roldan-Arjona T. et al., 1991 Klimisch 3
Reverse mutation and gene conversion No guideline GLP: no information	Vinylidene Chloride Purity: 99.57%	S. cerevisae strain D7 With and without metabolic activation (no more information) Concentrations: 10, 20, 30, 40, 50 mM Solvent: DMSO Negative control: yes	+ S9 mix: Positive - S9 mix: Negative	Bronzetti et al.,1981 Klimisch 3
Tests on mammalian cells				
MLA GLP: no information	Vinylidene Chloride Purity not provided	L5178Y cells With and without metabolic activation (from rat liver) Concentrations: 5 concentrations/trials (6 in one) up to 30% without S9 mix, up to 3.5% with S9 mix in suspension No vehicle Controls: yes. Positive: ethyl methanesulphonate, methyl methanesulfonate or 3-methylcholanthrene	Inconclusive without S9 mix Positive with S9 mix	Mc Gregor D. et al., 1991 Klimisch 2
MLA GLP: no information	Vinylidene Chloride (containing 0.3% 4-methoxyphenol	V79 cells With and without metabolic activation (from rat and mice liver) Concentrations: 2 or 10 % in air	Negative (+/- S9 mix) Cytotoxicity only with rat metabolic activation system	Drevon C. and Kuroki T. et al., 1979 Klimisch 3

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
	as antioxidant)	Negative controls treated the same way without the chemical No positive control		
UDS GLP: no information	Vinylidene Chloride Purity not provided	Freshly isolated rat hepatocytes With metabolic activation 2.1 mM Vehicle : ethanol Controls: negative: DMSO, positive: benzo(a)pyrene	Positive (+ S9 mix)	Costa A.K. et al., 1984 Klimisch 3
Chromosome Aberration Test Deviations: number of metaphases analysed per dose levels was insufficient (only 100 well-spread metaphases) GLP: no information	Vinylidene Chloride Purity: 99%	CHL (Chinese hamster cell) With and without metabolic activation (from rat liver) Concentrations: 0, 0.125, 0.250, 0.5, 1.0, 1.5, 2.0 mg/ml Vehicle : DMSO Negative control: yes Positive controls: no information No information on number of replicates.	- S9 mix: Negative + S9 mix: Positive	Sawada M. et al., 1987 Klimisch 3
Sister Chromatid Exchange Assay GLP: no information	Vinylidene Chloride Purity: 99%	CHL (Chinese hamster cell) With and without metabolic activation (from rat liver) Concentrations: 0; 0.025; 0.05; 0.075; 0.1 mg/mL Vehicle : DMSO Negative control: yes Positive controls: no information	- S9 mix: Negative + S9 mix: Positive No information on cytotoxicity	Sawada M. et al., 1987 Klimisch 3
Other tests				
Saccharomyces cerevisae, mitotic recombination assay GLP: no information	Vinylidene Chloride Analytical grade Purity not provided	Strains D7 and D61.M With and without metabolic activation (from mouse liver) Concentrations: 25; 50; 75; 100 mM. Negative control: yes Positive control substances: ethylmethanesulphonate (for D7 strain) and ethylacetate (for D61.M strain)	Strain D7 : Positive (+ S9 mix) Strain D61.M : Positive (+/- S9 mix)	Koch R. et al., 1988 Klimisch 2
Detection of one single-strand break GLP: no	Vinylidene Chloride Purity not provided	Double-stranded circular phage PM2 DNA molecule No information on concentrations and controls	Positive	Waskell, 1978 Klimisch 4

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
information				
Mitotic chromosome malsegregation GLP: no information	Vinylidene Chloride Purity: 99%	Aspergillus nidulans Concentrations: 0.025; 0.05; 0.1; 0.125; 0.15; 0.175; 0.2 % v/v Vehicle : DMSO Untreated control included Positive control: thiabendazole	Positive	Crebelli R. et al., 1992 Klimisch 4

[a]The reports of these studies were not available to the DS but they are summarised on ECHA disseminated website. Results were also available from other secondary bibliographic sources.

Table 15: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Comet assay in lung, liver, kidney and bone marrow cells According to OECD guideline 489 GLP : yes	Vinylidene Chloride Purity > 99.9%	5 male Wistar Han rats/group 25, 250, 750 and 6350 ppm (100, 1000, 3000 and 25000 mg/m^3) by inhalation (nose only) 4h per day for 3 days. Cells recovered between 2 and 6 hours after the last exposure. Negative control: similarly exposed to air Positive control: ethylmethanesulphonate (EMS) 200 mg/kg bw by gavage	Statistically significant increase in DNA damage in lung, liver and kidney No DNA damage observed in bone marrow cells No mortality. Histopathological findings reported in the lung, liver and kidneys, mainly at the highest dose.	Anonymous, 2016 Klimisch 1
Micronucleus in bone marrow in mice Equivalent or similar to OECD guideline 474 GLP: no information	Vinylidene Chloride Purity: 99%	Male mice ddY Gavage Single administration or multiple administration (x 4; 24h interval) 0; 25; 50; 100; 200 mg/kg bw 6 males/dose Negative control: vehicle (olive oil) Positive control: Mitomycin C (IP injection)	Toxicity: 3 out of 6 animals died after a single treatment with 200 mg/kg, and 1 animal died after 4 treatments with 100 mg/kg. No significant changes observed in the ratio of PCE to total erythrocytes. No increase of micronucleated erythrocytes in bone marrow	Sawada M. et al., 1987 Klimisch 2
Micronucleus in peripheral blood in mice Similar to OECD guideline 474 GLP: yes	Vinylidene Chloride Purity > 99.9%	5 mice B6C3F1/sex/dose Whole body inhalation 6h/d, 5 d/week for 14 weeks. 6.25, 12.5, 25, 50 ppm (both sexes), 100 ppm (females only) 5 mice/sex/dose	Toxicity: 2 males at 50 ppm and 4 females at 100 ppm died, decreased body weight in all exposed females and males exposed to 12.5 ppm or greater, haematological changes from 12.5 ppm in males. No change in the percentage of	NTP, 2015 Klimisch 2

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
		Negative control: air No positive control	PCE seen. No increase in the frequency of micronucleated erythrocytes observed in peripheral blood of B6C3F1 mice	
Micronucleus in fetal liver and blood in mice No guideline available GLP: no information	Vinylidene Chloride Purity: 99%	Pregnant female mouse ICR Single IP administration (GD 18) 25; 50; 100 mg/kg 4 fetuses for control and 25 mg/kg 6 fetuses for 50 and 100 mg/kg Negative control: vehicle (olive oil) Positive control: no data	Toxicity: not specified. The ratio PCE/(PCE + NCE) did not show any sign of cytotoxicity. Negative	Sawada M. et al., 1987 Klimisch 3
Chromosome aberrations in bone marrow in rats Similar to OECD guideline 475 Deviations: only 2 doses and 4 animals. Only 50 metaphases (instead of 200 as required in the guideline) analysed per animal and no details on the type of aberrations. No mitotic index calculated to assay the cytotoxicity to bone marrow and justify its exposure. GLP : not specified	Vinylidene Chloride Purity: 99%	Male and female Sprague-Dawley rats Exposure: 6h/d 5 days/week for 6 months Inhalation; whole body 0, 25, or 75 ppm (100 and 300 mg/m^3) 4 rats/sex/group Positive control: no data	No information on toxicity. No chromatid or chromosomal aberrations in VDC-exposed groups of rats.	Quast et al., 1986 Klimisch 3
Dominant Lethal Test Similar to OECD guideline 478 GLP: not specified Test conditions not sufficiently detailed, individual results not available	Vinylidene Chloride Purity not provided	CD-1 male mice 6 h/day, 5 days. Sacrifice of females on GD15. Inhalation 10, 30, and 50 ppm 20 exposed males/group 50 control males (15 air, 35 housed under normal conditions) Positive control: cyclophosphamide, 200 mg/kg bw once by IP injection on day 5	No evidence of a mutagenic effect with VDC Mating frequency high in the two groups exposed to the lowest doses of VDC in all weeks by comparison with the negative control group, and statistically significantly lowers in the high exposure group in weeks 0-8 and the positive control.	Anderson D. et al., 1977 Klimisch 2
Dominant Lethal Test	Vinylidene	11 CD male rats	VDC exposure did not produce dominant lethal mutations in	Short R.D. et

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Similar to OECD guideline 478 Deviations: only one dose tested, number of animals per group insufficient, test conditions not sufficiently detailed GLP: not specified	Chloride Purity not provided	6 h/d for 5 d/wk for 11 weeks + days of mating. Sacrifice of females on GD13. Inhalation 55 ppm (220 mg/m^3) Negative control: air No positive control	the germinal cells of male rats. Reduced ratio of pregnant to mated females observed (considered to be due to pre-implantation loss).	al., 1977b Klimisch 3
DNA synthesis and DNA repair No guideline GLP: not specified	Vinylidene Chloride Purity: 99.95%	Males CD-1 mice and Sprague-Dawley rats Exposure by whole body inhalation 6 hours 10 (both species) and 50 ppm (mice only) Controls: negative and positive (Dimethylnitrosamine, DMN)	Alkylated DNA recovered from the livers and kidneys. However, compared with animals exposed to IP injection of DMN, few alkylated nucleotides recovered and DNA repair synthesis only modestly elevated. Histopathological findings mainly in the kidney at 50 ppm.	Reitz R.H., et al., 1980 Klimisch 3
Sex-linked recessive lethal assay Similar to OECD Guideline 477 (deleted in 2014) GLP: not specified	Vinylidene Chloride Purity: 98%	Drosophila melanogaster males 3 day feeding exposure: 20,000; 25,000 ppm in 5% sucrose solution (Sealed vials) If the results of the feeding SLRL test were negative, an injection exposure was performed: 5,000 ppm in ethanol Toxicity tests performed to set concentrations of VDC at a level that would induce 30% mortality after 72h of feeding or 24h after injection. Negative control: yes No positive control	No increase in sex-linked recessive lethal mutations	Foureman P., et al., 1994 Klimisch 4

Table 16: Summary table of mutagenicity/genotoxicity other tests

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Reverse mutation and gene conversion, host-mediated assay No guideline GLP: no information	Vinylidene Chloride Purity: 99.57%	S. cerevisae strain D7 Concentrations: 400 mg/kg bw, single oral dose; 100 mg/kg bw/d for 5 d/wk, total of 23 dosings Solvent: DMSO Negative control: yes (no more information)	Point mutations and mitotic gene conversion seen in the yeast recovered from kidney and liver, but not lung	Bronzetti et al., 1981 Klimisch 3

10.6.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

Genotoxicity assessment of vinylidene chloride has been subject to numerous assays, either *in vitro* or *in vivo*.

In vitro

Numerous gene mutation studies in bacteria have been performed with VDC. Many of these studies are old, have methodological limitations and are not sufficiently detailed, but still allow to draw a genotoxic profile of VDC. In particular, two of them appear of better quality (Mortelmans et al., 1986 and Oesch et al., 1983) comparing to the dataset. From these two Ames tests, contradictory results were obtained with VDC being mutagenic in S. typhimurium strains TA1535, 1537, 98, 100 and 92 and E. Coli WP2 in presence of metabolic activation only (Oesch et al., 1983) and not mutagenic with and without metabolic activation in S. typhimurium strains TA1535, 1537, 98, 100 (Mortelmans et al., 1986), but with no apparent reason to explain these discrepancies.

Overall, VDC is mutagenic in different strains (S. thyphimurium TA98, TA100, TA1535, TA1530, BA13, BAL13, TA1537, TA1538, TA92, E. coli K-12, E. coli WP2, depending on the study considered) generally in the presence of an exogenous metabolising system, either from liver, kidney or lung, from mice or rats (Simmon et al. 1977, Waskell, 1978, Greim et al. 1975, Bartsch et al. 1975, Roldan-Arjona et al. 1991, Baden et al. 1978 and 1982, Jones & Hathway 1978b; Oesch et al. 1983).

VDC has been shown to be much more mutagenic in strain TA1535 in the presence of induced than uninduced mouse liver and kidney S9, whereas VDC is only mutagenic in the presence of induced rat liver S9 (Jones and Hathway, 1978b). This demonstrates the greater sensitivity of the mouse species to VDC mutagenesis. In a study comparing mutagenicity of chlorinated ethylenes, VDC exert a small but definite mutagenic effect in the presence of induced mouse liver on E. Coli K12 (Greim et al., 1975).

However, in two other Ames tests (the study of Mortelmans et al., 1986 cited earlier and Laumbach et al. 1977), VDC showed no mutagenic activity in strains TA98, TA100, TA1535 and TA1537 with and without induced rat or hamster liver S9.

In yeast models, S. cerevisiae D7, VDC is mutagenic only in the presence of induced mouse liver S9, increasing the rate of gene mutations and gene conversions (Koch et al., 1988; Bronzetti et al., 1981). VDC also induces aneuploidy in S. cerevisiae D7 in the absence of S9 as visualised by chromosome mis-segregation (Koch et al., 1988).

In mammalian cells, VDC has not been shown to induce gene mutations in V79 cells with and without metabolic activation system (from rat and mice liver) (Drevon and Kuroki., 1979). However, it is mutagenic in the MLA tk+/- test on L5178Y cells with S9 from induced rat liver, but with equivocal results (contradictory findings between 3 trials) without S9 (Mc Gregor et al., 1991).

VDC has been shown to induce unscheduled DNA repair in rat hepatocytes in primary culture (Costa et al., 1984). VDC also induces single-strand breaks in phage PM2 after 62 hours incubation (Waskell L., 1978).

VDC induces chromosomal aberrations and sister chromatid exchanges (relatively weak (1.6/1.8 fold), but significant increase) in CHL cells only in the presence of induced rat liver S9 (Sawada et al., 1987). To be noted that in chromosomal aberrations study, metyrapone (an inhibitor of the P-450 activity) was added to the culture at several concentrations together with S9 mix. Authors reported that the frequencies of aberrant cells decreased as the concentration of metyrapone increased (0.1-1.0 mM). This finding confirms that cytochrome P-450 in the liver microsome participates in the activation of VDC.

In conclusion, VDC can be considered as a mutagenic compound that induces gene and chromosome mutations *in vitro* in the presence of an exogenous metabolising system.

In vivo

Genotoxicity of VDC was investigated in several *in vivo* studies.

A comet assay was performed according to OECD test guideline 489 in male Wistar Han rats exposed by nose only inhalation to VDC at 25, 250, 750 and 6350 ppm (corresponding to 0.1, 1, 3 and 25 mg/L) 4 hours per day for 3 days. Lung, liver, kidney and bone marrow cells were recovered between 2 and 6 hours after the last exposure. In this assay, VDC induced a statistically significant increase in DNA damage in lung, liver and kidney (at all concentrations in the kidney and the lung and from 1 mg/L in the liver) (Anonymous, 2016). Some histopathological lesions (from minimal to severe severity) were also observed in these organs (at the highest dose in kidney, the two highest doses in lung, and the three highest doses in liver), but, in accordance with the OECD guideline, this does not question the relevance of the DNA damages observed, taking also into account the fact that the observed DNA damages occurred at concentrations below or concomitantly to those inducing histopathological findings. A trend test was not performed by the authors to assess if the DNA damages were concentration related, but it was probably not possible as the negative controls associated to each concentration were different. It can also be noted that the observation of DNA damages in these organs (containing enzymes of metabolism) is consistent with the fact that VDC is extensively metabolised into genotoxic metabolites. No DNA damages were seen in bone marrow. However, in the absence of evidence of bone marrow exposure, these results are not usable to confirm the absence of genotoxic potential of VDC. Furthermore, given the extensive hepatic metabolism of VDC to potentially genotoxic metabolites as demonstrated *in vitro,* it is unlikely that these highly reactive metabolites would be able to reach the bone marrow.

The various micronucleus tests on bone marrow cells or circulating mouse erythrocytes are negative, as well as the chromosomal aberration test on rat bone marrow cells (tests performed after inhalation or gavage exposure) (Sawada et al., 1987; NTP, 2015; Quast et al., 1986). In these studies, there was no proof that the target organ, i.e the bone marrow, was adequately exposed (no to slight toxicity, no change in the percentage of PCE/NCE). As for the comet assay, negative results on bone marrow cells cannot be used to demonstrate the absence of genotoxicity. In addition, most of the studies did not report the inclusion of a positive control to validate the results (Sawada et al. 1987; NTP, 2015; Quast et al., 1986).

Two dominant lethal mutation tests were performed in mice and rats and failed to show the ability of VDC to induce mutations in male germ cells after inhalation exposures (Short et al., 1977b; Anderson et al., 1977). However, the study of Short et al. (1977b), has important limitations, as the use of only one concentration of exposure or the absence of positive control which preclude the use of the results.

A sex-linked recessive lethal mutation test in Drosophila melanogaster showed no mutagenic effects of VDC (Foureman P. et al., 1994). Few informations on protocol are however available to assess the study.

In a study where CD-1 mice and Sprague-Dawley rats were exposed by inhalation to 10 or 50 ppm radiolabelled VDC for 6 hours, alkylated DNA was recovered from the livers and kidneys. However, compared with animals exposed to intraperitoneal injection of dimethylnitrosamine, a known mutagen, few alkylated nucleotides were recovered and DNA repair synthesis was only modestly elevated (Reitz et al., 1980).

The *in vivo* genotoxicity dataset show that VDC is able to cause primary DNA damage both at the site of contact, the lung and in peripheral organs, the liver and the kidney (the two later being main organs of metabolisation, in coherence with in vitro genotoxicity results and toxicokinetics data). However, VDC does not induce genotoxic effects on bone marrow cells or male germ cells in available studies, with the limitations described above.

Overall, *in vitro* results are mainly positive with metabolic activation. *In vivo*, only the well-conducted and recent comet assay is positive. The majority of *in vitro* assays available point to the induction of gene mutations (as the Ames test). In contrast, in the *in vivo* assays available, only the comet assay is able to detect gene mutation and returns positive, confirming the concern raised *in vitro*. This assay is positive in lung, i.e. at the site of contact, and liver and kidney, which is consistent with the proposed metabolic pathway of VDC generating mutagen metabolites (such as epoxides) and with the *in vitro* observations where results are mainly positive with metabolic activation. Results on bone marrow cells (from the Comet assay and micronucleus assays) are consistently negative. Nevertheless, there are some doubts on the adequate exposure of this organ. The other *in vivo* assays only investigate clastogenicity and aneuploidy. The negative results in these assays can be explained by the cells investigated, as described above: without proof of reaching the tissues, these results cannot be used to demonstrate the absence of genotoxicity.

10.6.2 Comparison with the CLP criteria

According to CLP criteria, "*For the purpose of classification for germ cell mutagenicity, substances are allocated to one of two categories*"

Category 1: "*Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans*."

- Category 1A: "*The classification in Category 1A is based on positive evidence from human epidemiological studies*."

No epidemiological data are available concerning the mutagenic potency of VDC. Category 1A can therefore not be considered.

- Category 1B: "*The classification in Category 1B is based on*:
    - *positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or*
    - *positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or*
    - *positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.*"

As suggested in the criteria, distinction needs to be made between 'mutagenicity tests' in the strict sense and 'indicator tests' that provide evidence of interaction with DNA that may or may not lead to mutations (e.g. DNA adducts, DNA strand breaks and sister chromatid exchanges). Preference should be given to mutagenicity tests whenever possible.

Regarding exclusively the *in vivo* dataset on VDC, no positive results from the mutagenicity tests in the strict sense (micronucleus tests, chromosomal aberrations tests or dominant/sex-linked recessive lethal mutation tests) are shown, either on somatic or germ cells. It should be noted however that concerning the micronucleus and chromosomal aberrations tests, there is no evidence that VDC reaches the bone marrow, meaning that these studies cannot be used to conclude on the absence of genotoxicity of VDC. Moreover, most of the *in vitro* assays point to the induction of gene mutations while *in vivo* mutagenicity studies available with VDC can only detect chromosomal aberrations. The positive Comet assay (Anonymous, 2016), as explained by WHO (2020) "*is an indicator test for genotoxicity, as there are multiple fates of the DNA damage detected in this assay: accurate repair of the damage, cell death due to inability to repair, or incorrect repair, which may lead to mutation or chromosomal damage (i.e. permanent, viable, heritable change). Hence, there may be no heritable consequences of a positive finding in this assay*" and can therefore not be used as a stand alone to justify a classification in category 1B. However, it should be noted that the NTP study (2015) performed on mice showed effects of VDC through inhalation on epididyme and sperm, showing that the substance reaches the reproductive organs.

Category 2: "*Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:*

- *Somatic cell mutagenicity tests in vivo, in mammals; or*
- *Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays."*

In vivo, in a recent well-performed Comet assay performed according to OECD guideline 489, VDC induces DNA damage after inhalation in male rats in the lungs, liver and kidneys (Anonymous, 2016).

In vitro, VDC demonstrates its mutagenic potency mostly in the presence of an exogenous metabolic system in numerous mutagenicity assays, in particular Ames tests (Simmon et al., 1977; Waskell, 1978; Greim et al., 1975; Bartsch et al., 1975; Roldan-Arjona et al., 1991; Oesch et al., 1983; Jones and Hathway, 1978b; Bronzetti et al., 1981; Koch et al., 1988). These results support the positive findings observed in the *in vivo* Comet assay, in particular since the liver, the kidney and the lung express several enzymes involved in the metabolism of VDC to mutagen compounds (such as epoxides).

Therefore, VDC is mutagen in 1) *in vivo* somatic cell genotoxicity test and 2) *in vitro* mutagenicity assays. Criteria for a classification in category 2 are therefore fulfilled.

10.6.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on the data available, a classification as **Muta. 2, H341: Suspected of causing genetic defects** is warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The data set for mutagenicity was quite extensive and it was evaluated thoroughly by the DS.

In vivo, there was a recent well-performed Comet assay of high reliability, conducted according to OECD TG 489. It demonstrated that VDC induced DNA damage in the lungs, liver and kidneys of male rats, after inhalation.

In vitro, VDC was found to be mutagenic in different strains (S. thyphimurium TA98, TA100, TA1535, TA1530, BA13, BAL13, TA1537, TA1538, TA92, E. coli K-12, E. coli WP2, depending on the study considered) generally in the presence of an exogenous metabolising system (metabolic activation), either from liver, kidney or lung of mice or rats. VDC was also shown to be mutagenic in yeast models in the presence of induced mouse liver S9, increasing the rate of gene mutations and gene conversions. In mammalian cells, VDC was shown to be mutagenic in the MLA tk+/- test on L5178Y cells with S9 from induced rat liver. These results supported the positive findings observed in the *in vivo* Comet assay, especially since the liver, the kidney and the lung express several enzymes involved in the metabolism of VDC to mutagenic metabolites, such as epoxides, as explained in the toxicokinetics section.

In conclusion, VDC was found to be mutagenic in an *in vivo* somatic cell genotoxicity test and in various *in vitro* mutagenicity assays. Therefore, the criteria for a classification in Category 2 were considered fulfilled. Consequently, the DS proposed classification as Muta. 2; H341: Suspected of causing genetic defects.

Comments received during consultation

Two comments were received.

One MSCA supported the proposed classification and reasoning by the DS.

One comment was received from an Industry/Trade Association disagreeing with the proposed classification. While the commenting Industry/Trade Association acknowledged the positive *in vitro* results as well as the positive *in vivo* Comet assay for VDC, the following arguments were presented:

- Four reliable (Klimisch 2 and 3) studies (*in vivo* micronucleus tests, chromosomal aberration test) were negative: the *in vivo* micronucleus tests on bone marrow or circulating erythrocytes conducted in mice and the chromosomal aberration test on rat bone marrow cells did not show any evidence of chromosomal aberrations.
- Even though VDC was shown to reach the bone marrow in one of the above-mentioned micronucleus tests, it did not induce any genotoxic effects.
- Two dominant lethal (DL) assays showed negative results.
- A Sex-Linked Recessive Lethal Mutation *in vitro* assay on Drosophilia melanogaster was negative.

Hence, the Industry/Trade Association concluded that VDC was not expected to induce heritable genetic damage (chromosomal aberrations or gene mutations) and considered that classification was not warranted in accordance with the CLP Regulation.

The DS replied that according to the CLP Regulation where there is evidence of only somatic cell genotoxicity, substances should be classified as suspected germ cell mutagens (i.e., Category 2). Classification as a suspected germ cell mutagen may also have implications for potential carcinogenicity classification. This was considered to hold true especially for those genotoxicants which were incapable of causing heritable mutations because they could not reach the germ cells (e.g., genotoxicants only acting locally, 'site of contact' genotoxicants). This was considered to be the case for VDC where the genotoxic effects *in vivo* were seen locally only in detoxification organs. The DS clarified that if positive results *in vitro* were supported by at least one positive local in vivo somatic cell test, such effects should be considered as sufficient justification to classification in Category 2.

Moreover, the DS added that the negative *in* vivo studies did not investigate the same endpoints and the same organs as the comet assay, and this could according to the DS explain the results observed. A comet assay is the only assay allowing the identification of site of contact genotoxicants.

Assessment and comparison with the classification criteria

All the available *in vitro* and *in vivo* studies presented in Tables 14, 15 and 16 (pages 23-29) of the CLH report were evaluated by RAC.

In vitro

In bacterial test systems, VDC consistently demonstrated mutagenic activity when tested in the presence of a metabolic activation system, in a closed environment to control volatility.

Many of these studies were old, had methodological limitations and were not reported in adequate detail, but still allowed to assess a genotoxic profile of VDC.

VDC was shown to be mutagenic in different strains (S. thyphimurium TA98, TA100, TA1535, TA1530, BA13, BAL13, TA1537, TA1538, TA92, E. coli K-12, E. coli WP2), generally in the presence of an exogenous metabolising system either from liver, kidney or lung from mice or rats.

Mutagenicity was higher in the presence of liver mouse S9, but lower when mouse kidney or lung S9 fractions were used (Bartsch *et al*., 1975). Phenobarbital, a potent cytochrome P450 inducer, in accordance with the analysis in the toxicokinetics section, increased mutagenic responses in tests using mouse liver, kidney, and lung S9 (Bartsch *et al*., 1975). Similarly, VDC was mutagenic in strains TA100 and TA1535 (Baden *et al*., 1978, 1982), in which pre-treatment with CYP inducers increased the effectiveness of mouse liver and kidney S9, with mouse liver S9 also being more effective than rat liver S9.

Positive results were also observed in S. typhimurium strains TA92, TA98, TA100, TA1535, and TA1537 and E. coli strain WP2 uvrA in the presence of human S9 or Swiss mouse liver S9 (Oesch *et al*., 1983).

In yeast models, S. cerevisiae D7, VDC was mutagenic only in the presence of induced mouse liver S9, increasing the rate of gene mutations and gene conversions (Koch *et al*., 1988; Bronzetti *et al*., 1981). VDC also induced aneuploidy in S. cerevisiae D7 in the absence of S9 as visualised by chromosome malsegregation (Koch *et al*., 1988).

On the contrary, VDC (tested at up to 6666 µg/plate) was not mutagenic in strains TA98, TA100, TA1535, or TA1537, with or without induced S9, when a preincubation protocol was used (Mortelmans *et al*., 1986). However, there are uncertainties on whether the conditions used in this specific study could completely account for the high volatility of VDC.

In mammalian systems inconsistent mutagenic responses were seen in L5178Y mouse lymphoma cells with VDC in the absence of metabolic activation; with activation, both cytotoxicity and mutagenicity were consistently positive in several experiments.

VDC did not induce gene mutations in V79 cells with or without metabolic activation from rat and mice liver. VDC stimulated unscheduled DNA synthesis in isolated rat hepatocytes in primary culture. VDC also induced single-strand breaks in phage PM2 DNA after 62-h incubation.

Strong, dose-related increases in chromosomal aberrations were seen in cultured Chinese hamster lung (CHL) cells exposed to VDC in the presence of induced rat liver S9. In addition, VDC induced relatively weak sister chromatid exchanges (1.6/1.8-fold), but significant increase in CHL cells only in the presence of induced rat liver S9. It is noted that in the chromosomal aberration study, metyrapone (an inhibitor of the P-450 activity) was added to the culture at various concentrations together with S9 mix. The authors reported that the frequencies of aberrant cells decreased as the concentration of metyrapone increased (0.1-1.0 mM), thus, confirming that cytochrome P-450 in the liver microsome participates in the activation of VDC.

In vivo

The mutagenicity/genotoxicity of VDC has been investigated in several *in vivo* studies. The most recent and reliable study is a comet assay performed according to OECD TG 489 in male Wistar Han rats exposed by nose-only inhalation to VDC at 25, 250, 750 and 6350 ppm (corresponding to 0.1, 1, 3 and 25 mg/L) for 4 hours per day for 3 days (Anonymous 2016,

Klimisch 1). Lung, liver, kidney and bone marrow cells were recovered between 2 and 6 hours after the last exposure. In the following table, data on viability of single cell suspensions is provided:

***Table**: Sigle cell suspensions viability in the comet assay in Anonymous (2016)*

Animal #	Dose	Viability (%)			
		Kidney	*Liver*	*Lung*	*Bone Marrow*
1	0	98	100	100	98
6	25 mg/L	99	99	100	96
11	200 mg/kg (EMS)	96	100	100	99
16	0	95	100	100	95
21	25 mg/L	94	100	99	97
26	200 mg/kg (EMS)	99	98	100	92
31	0	100	100	100	96
36	25 mg/L	97	98	97	98
41	200 mg/kg (EMS)	100	100	98	98
46	0	100	100	100	100
51	25 mg/L	100	100	100	100
56	200 mg/kg (EMS)	100	100	100	84

In this assay, VDC induced a statistically significant increase in DNA damage in lung, liver and kidney (at all concentrations in the kidney and the lung and from 1 mg/L in the liver). Results are summarised in the following table.

***Table**: Histopathology and DNA damage results in the comet assay in Anonymous (2016)*

Organ	Dose (mg/L)	Histopathology Findings		Comet Assay (DNA Damage)			
		Severity	Description	Relevance	Tail Intensity (SD)		
					Treated Group	Negative Control	Positive Control
Lung	25	Severe	Degeneration/regeneration bronchiolar epithelium Lymphoid depletion BALT Inflammatory cell infiltrate peribronchial	Relevant	32.0 (24.4)*	4.61 (0.69)	87.0 (12.2)***
	3	Minimal	Regeneration bronchiolar epithelium Inflammatory cell infiltrate peribronchial	Relevant	50.0 (7.39)***	19.7 (9.75)	93.4 (1.80)***

	1	-	-	Relevant	81.6 (3.31)***	15.0 (7.08)	90.6 (2.81)***
	0.1	-	-	Not relevant	5.65 (1.38)	4.73 (1.01)	82.6 (3.43)***
Liver	25	Severe	Single cell necrosis, centrilobular/ bridging	Relevant	20.0 (7.94)***	3.65 (3.34)	92.7 (4.80)***
	3	Severe	Single cell necrosis, centrilobular/ bridging Hepatocellular hypertrophy, centrilobular	Relevant	33.3 (8.93)***	15.4 (4.30)	99.0 (0.21)***
	1	Severe	Single cell necrosis, centrilobular/ bridging Hepatocellular hypertrophy, centrilobular	Relevant	44.4 (16.9)*	16.4 (14.7)	94.5 (1.62)***
	0.1	Not adverse	Hepatocellular hypertrophy, centrilobular Cytoplasmic alteration centrilobular	Not relevant	16.9 (4.08)	14.4 (5.50)	86.8 (2.86)***
Kidney	25	Severe	Tubular degeneration	Relevant	68.7 (13.8)***	4.37 (0.76)	97.0 (1.55)***
	3	-	-	Relevant	57.2 (2.56)***	11.6 (2.56)	96.6 (1.89)***
	1	-	-	Relevant	35.6 (7.90)*	26.6 (5.07)	92.9 (1.09)***
	0.1	-	-	Relevant	55.0 (11.0)***	13.8 (3.52)	87.2 (3.24)***
Bone Marrow	25	-	-	Not Relevant	4.00 (0.95)	4.32 (2.08)	80.3 (10.0)***
	3	-	-	Questionable	11.6 (3.18)**	5.31 (1.30)	85.4 (2.81)***
	1	-	-	Not Relevant	11.9 (4.42)	10.2 (1.99)	82.2 (2.56)***
	0.1	-	-	Not Relevant	12.8 (7.10)	10.0 (3.02)	78.2 (3.16)***

** p<0.05; ** p<0.01; *** p<0.001 (Student t test)*

Histopathological lesions (from minimal to severe in severity) were also observed in these organs (at the highest dose in kidney, the two highest doses in lung, and the three highest doses in liver), but, in accordance with the OECD TG, this does not confound the relevance of the DNA damages observed, taking also into account the fact that the observed DNA damages occurred also at concentrations below to those inducing histopathological findings. A trend test

was not performed by the authors to assess if the DNA damages were concentration related. It is noted, that for each tested concentration different negative controls were used. DNA damages observed in liver, kidney and lung are consistent with the fact that VDC is extensively metabolised into genotoxic metabolites in these tissues. In contrast, no DNA damage was seen in bone marrow. However, under these experimental conditions bone marrow exposure to VDC was not confirmed by the study authors.

In the Sawada *et al*. (1987) micronucleus study, there was a 23% decrease in the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (PCE/NCE ratio) at 200 mg/kg indicating that under the conditions of the specific study (i.e., gavage administration) the bone marrow of male mice was probably exposed to VDC. It is noted that in the OECD TG 474 Mammalian Erythrocyte Micronucleus Test, "the highest dose may also be defined as a dose that produces toxicity in the bone marrow (e.g., a reduction in the proportion of immature erythrocytes among total erythrocytes in the bone marrow or peripheral blood of more than 50%, but to not less than 20% of the control value)".

In other *in vivo* studies no evidence of genotoxicity was seen after VDC exposure. The various micronucleus tests on bone marrow cells or circulating mouse erythrocytes were negative, as well as the chromosomal aberration test on rat bone marrow cells (i.p. administration in Sawada *et al*., 1987; whole-body inhalation exposure in NTP, 2015 and in Quast *et al*., 1986).

In these studies, there was no evidence that the target organ, i.e., the bone marrow, was adequately exposed (lack of/slight toxicity, no change in the percentage of PCE/NCE). Therefore, as previously discussed for the comet assay, negative results on bone marrow cells cannot be used to demonstrate the absence of genotoxicity. In addition, most of the studies did not report the inclusion of a positive control to validate the results.

Negative results were also reported in dominant lethal tests (assays for mutagenicity in germ cells) in male CD-1 mice (Anderson *et al*., 1977) and in male Crl:CD(SD) rats exposed to VDC by inhalation (Short *et al*., 1977).

VDC did not induce increases in sex-linked recessive lethal mutations in germ cells of adult male Drosophila melanogaster exposed via feed or injection (Foureman *et al*., 1994). However, the study had several reporting deficiencies.

Given the extensive hepatic metabolism of VDC to potentially genotoxic metabolites as suggested by the results of the *in vitro* tests, it is unlikely that these highly reactive metabolites would be able to reach the bone marrow or the germ cells via the blood stream, thus explaining most of the negative results presented above.

Although in the available toxicokinetic studies there is no evidence that the substance or its reactive metabolites reach and interact with the genetic material of germ cells, in the subchronic inhalation NTP study (2015), it was shown that VDC had effects on the male reproductive system both in rats and mice. More specifically, spermatid heads (106/g testis) decreased 14.6% at 100 ppm, while at 25 ppm a decrease of 9.18% is recorded. Similarly, sperm motility was decreased by 2.5-4.5%. However, RAC is of the opinion that these results do not constitute robust evidence of the ability of VDC to interact with germ cells.

In conclusion, the table below summarises the *in vitro* and in *vivo* studies rendering positive results.

Table: *Summary of positive results in the mutagenicity battery of tests*

Test Organism	Test System		Endpoint
In vitro			
Bacteria	TA1535, TA1537, TA98, TA100 and TA92	+ S9	Reverse gene mutation
Bacteria	E. coli WP2	+ S9	Reverse gene mutation
Bacteria	E. coli K-12	+ S9	Reverse gene mutation
Bacteria	BA13 (mutation indicator) and BAL13 (survival indicator)	+ S9	Forward gene mutation
Yeast	S. cerevisae strain D7	+ S9	Reverse gene mutation
Yeast	S. cerevisae strain D7	+ S9	Gene Conversion
Yeast	D61.M	+/- S9	Chromosomal aberration (Aneuploidy)
Mammalian	L5178Y cells	+ S9	Forward gene mutation
Mammalian	Rat hepatocytes	- S9	DNA damage/repair (UDS)
Mammalian	Chinese hamster, lung	+ S9	Chromosomal damage (CA test)
Mammalian	Chinese hamster, lung	+ S9	Chromosomal damage (SCE assay)
Bacteria	Double-stranded circular phage PM2 DNA molecule	+ S9	DNA damage/repair (single-strand break)
In vivo			
Male Wistar Han rats	Comet assay in lung, liver, kidney and bone marrow cells	-	DNA damage

Several of the available *in vitro* studies were positive in the presence of exogenous metabolic activation and provide evidence for the mutagenic properties of VDC. VDC induced gene mutations in bacteria systems, yeast models and in mouse lymphoma cells, it was positive in a UDS test in rat hepatocytes, induced chromosomal aberrations and sister chromatid exchanges in Chinese hamster lung cells and induced aneuploidy in the presence and absence of metabolic activation in a single study in Saccharomyces cerevisiae.

In contrast, in the available *in vivo* assays, gene mutation was detected only in the comet assay, supporting the concern raised by the results of the *in vitro* studies. This assay was positive in lung (site of contact), liver and kidney. These findings are consistent with the proposed metabolic pathway of VDC generating mutagenic metabolites (such as epoxides) and the excretion pattern followed. In addition, the positive *in vitro* mutagenic results obtained only in the presence of metabolic activation support the mutagenicity of VDC metabolites.

Comet assay is a test which identifies chemicals that induce primary DNA damage. Under

alkaline conditions (> pH 13), the comet assay can detect single and double strand breaks resulting, for example, from direct interactions with DNA alkali labile sites, as a consequence of transient DNA strand discontinuities resulting from DNA excision repair, or from processing during the assay. These DNA strand breaks may be: 1) repaired, resulting in no persistent effect; 2) lethal to the cell; or 3) fixed as a mutation resulting in a permanent heritable change. Therefore, the alkaline comet assay detects primary DNA strand breaks that do not always lead to gene mutations and/or chromosomal aberrations and cannot provide conclusive evidence for classification in Category 1B on its own.

In the *in vivo* dataset of VDC, there are no other positive results in either somatic or germ cells (micronucleus tests, chromosomal aberrations tests or dominant/sex-linked recessive lethal mutation tests). VDC did not induce genotoxic effects in bone marrow cells or male germ cells. However, as already mentioned it is not certain that the substance reached these target tissues.

Regarding classification of VDC for mutagenicity, classification in Category 1A is not warranted based on the absence of human data.

A classification in Category 1B is not justified because no *in vivo* heritable mutagenicity studies in mammals or positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells, are available.

VDC was found to fulfil the CLP criteria for a classification in Category 2 based on:

- in an *in vivo* somatic cell (lungs, liver and kidneys) genotoxicity test (comet assay performed according to OECD TG 489)
- in various *in vitro* mutagenicity tests (mainly Ames tests) mostly in the presence of metabolic activation

In conclusion, RAC considers that **classification as Muta. 2; H341: Suspected of causing genetic defects is warranted.**

10.7 Carcinogenicity

Table 17: Summary table of animal studies on carcinogenicity by inhalation route

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Studies in rats			
Similar to OECD 451 GLP compliant F344 rats 50 rats/sex/dose	Vinylidene chloride Purity > 99% 0, 25, 50, 100 ppm (0, 100, 200, 400 mg/m^3) Whole body exposure 6 h/d, 5 d/week for 105	The survival of exposed groups of males was similar to that of controls. The survival of females exposed at 100 ppm was significantly less than that of controls (19/50 vs 30/50 in control group). Mean body weights of exposed groups of male and female rats similar to those of controls. Males: incidences of **malignant mesothelioma** occurred with a significant positive trend and were significantly ↗ in all exposed groups compared with the control group (1/50, 12/50, 28/50, 23/50). Significant positive trend in the incidence of **adenoma of the nasal respiratory epithelium**	NTP, 2015 Klimisch 1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	weeks	(0/49, 0/50, 1/50, 4/50). **Renal tubule carcinomas** observed in rats exposed (0/50, 2/50, 1/49, 1/50). Females: **Malignant mesotheliomas** in one female exposed to the low concentration and one female exposed to the medium concentration. At the high concentration, **adenoma of the nasal respiratory epithelium** (1/50, 2%). Significantly ↗ incidences, with significant positive trends, seen for **C-cell adenoma of the thyroid gland** at the high concentration (3/50, 4/50, 6/48, 11/50), and for **C-cell carcinoma of the thyroid gland** at the low concentration (0/50, 6/50, 2/48, 2/50). Significantly ↗ incidences of **C-cell adenoma or carcinoma (combined) of the thyroid gland** at low and high concentrations (3/50, 10/50, 8/48, 13/50). Incidence of **mononuclear cell leukaemia** significantly ↗ at the high concentration, with a significant positive trend (10/50, 11/50, 13/50, 25/50).	
No guideline followed GLP: not stated SD rats 86 rats/sex/dose	Vinylidene chloride purity 99% 0, 10, 40 ppm for 5 weeks, then 0, 25, 75 ppm (0, 40, 160 and 0, 100 and 300 mg/m^3) Whole body exposure 6 h/d, 5 d/wk for 18 months Interim sacrifices at 1, 6 and 12 months Sacrifice at 24 months	No significant increase of tumours in males In females statistically ↗ incidence of **mammary gland adenocarcinoma** at low dose (2/84, 7/86, 4/84)	Rampy L.W. et al., 1977 / Quast J.F. et al, 1986 Klimisch 2
No guideline followed GLP: not stated Sprague-Dawley rats 60 or 54 females + 62 male and 61 female offspring exposed transplacentally from day 12 of gestation, and by inhalation postnatally with the same regimen as the breeders	Vinylidene chloride Purity > 99.9% 0, 100 ppm (0, 400 mg/m^3) Whole body exposure 4 h/d, 5 d/week for 7 weeks, then for 7 h/d, 5 d/week for 97 weeks	In breeders, non-significant increases in incidences of benign and malignant tumours of the mammary gland and malignant tumours of the mammary gland. Compared with controls, increased incidence of **leukaemia** found in exposed male (control, 12/158, 7.6%; exposed, 10/62, 16.1% [not significant]) and female (control, 1/149, 0.7%; exposed, 4/61, 6.5% [P < 0.03]) offspring.	Cotti et al., 1988 Klimisch 3
No guideline followed	Vinylidene chloride	No significant increase of tumours after the recovery period	Hong et al., 1981

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
GLP: not stated CD rats 4 to 16 rats/sex/dose Control groups: total of 36 control rats per sex	purity, 99% 0, 55 ppm (0, 220 mg/m^3) Whole body exposure 6 h/d, 5 days/week, for 1, 3, 6 or 10 months 12-months post-exposure recovery period		Klimisch 3
No guideline followed GLP: not stated SD rats 30 rats/sex/dose 100 rat/sex in control group Only the early results available. Except histology examinations for tumours analysis, no information on other examinations (hematology, clinical chemistry, urine analysis, body weight, organ weight…) or statistical methods available.	Vinylidene chloride Purity: 99.9% 0, 10, 25, 50, 100, 200-150 ppm (0, 40, 100, 200, 400, 800-600 mg/m^3) Whole body exposure 4 h/d, 4-5 days/week for 52 weeks	The highest dose level of 800 mg/m^3 was reduced to 600 mg/m^3 after 2 exposures because of high toxicity. ↗ in the incidence **of mammary tumours** observed in treated female rats, but without apparent dose-response trend	Maltoni C. et al., 1977 Maltoni C. et al., 1984 Klimisch 3
No guideline followed GLP: not stated CD rats 36 rats/sex/dose	Vinylidene chloride purity 99% 0, 55 ppm (220 mg/m^3) Whole body exposure 6 h/d, 5 d/week for 12 months Interim sacrifices of 4 rats after 1, 2, 3, 6, and 9 months	No significant increase of tumours	Lee C.C. et al., 1977 Klimisch 3
Studies in mice			
Similar to OECD 451 GLP compliant	Vinylidene chloride	Survival: Males: 29/50, 40/50, 32/50, 19/50. Females: 36/50, 25/50, 30/50, 24/50. Mean body weights of males exposed to the medium and high	NTP, 2015 Klimisch 1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
B6C3F1/N mice 50 mice/sex/dose	Purity > 99.9% 0, 6.25, 12.5, 25 ppm (0, 25, 50, 100 mg/m^3) 6 h/d, 5 d/week for 105 weeks	concentrations and females exposed to the high concentration were at least 10% lower than those of controls during the study. Males: Incidences of **renal tubule adenoma** (0/50, 5/50, 19/50, 10/50)**, renal tubule carcinoma** (0/50, 7/50, 31/50, 18/50)**, and renal tubule adenoma or carcinoma** (combined) (0/50, 11/50, 37/50, 27/50) significantly ↗ in all exposed groups, with a significant positive trend. Incidences of **renal tubule hyperplasia** significantly ↗ in all exposed groups. Incidences of **hepato-cholangiocarcinoma** in exposed groups non-significantly ↗ compared with that in the control group (1/50, 2/50, 2/50, 3/50) but exceeded the historical control range for inhalation studies (0–2%). Females: Incidences of **haemangioma of the vascular system** in all exposed groups non-significantly ↗ (0/50, 2/50, 2/50, 2/50) compared with controls. Significant positive trend in incidence **of haemangiosarcoma of the vascular system** (4/50, 4/50, 4/50, 9/50). Compared with controls, incidence of **haemangioma or haemangiosarcoma (combined) of the vascular system** (4/50, 6/50, 6/50, 11/50) significantly greater at high concentration, with a significant positive trend. Compared with controls, incidence of **liver haemangiosarcoma** (1/50, 1/50, 1/50, 6/50) significantly greater at high concentration, with a significant positive trend. Incidences of **hepatocellular adenoma** (medium concentration: 25/50, 21/50, 36/50, 29/50), **hepatocellular carcinoma** (high concentration: 8/50, 14/50, 12/50, 17/50), and **hepatocellular adenoma or carcinoma** (combined) (medium and high concentrations: 28/50, 30/50, 37/50, 38/50) significantly greater than in the control groups, with significant positive trends. **Hepato-cholangiocarcinoma** occurred in all exposed groups (0/50, 1/50, 1/50, 2/50). Incidence of **bronchioloalveolar carcinoma** significantly ↗ at medium concentration (1/50, 2/50, 7/50, 5/49) with a significant positive trend. At high dose, incidence of **carcinoma of the small intestine** (ileum) (3/50, 6%) exceeded historical control ranges.	
No guideline followed GLP: not stated Albino CD-1 mice 8-12 mice/sex/dose	vinylidene chloride Purity: 99% 0, 55 ppm (0, 220 mg/m^3) Whole body exposure 6 h/d, 5 days/week, 1, 3 and 6 months 12-months post-exposure recovery period	No significant increase of tumours	Hong C.B. et al., 1981 Klimisch 3
No guideline	Vinylidene	Study termination at 50, 100, 200 ppm due to high mortality	Maltoni C.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
followed GLP: not stated Swiss mice First part: control group: 100 mice/sex 30 mice/sex/dose Second part (only 25 ppm): control group: 90 mice/sex 25 ppm: 120 mice/sex	chloride Purity > 99.9% 0, 10, 25, 50, 100, 200 ppm (0, 40, 100, 200, 400, 800 mg/m^3) Whole body exposure 4 h/d, 4-5 days/week for 52 weeks Observation up to 121 weeks	and severe toxicity observed. First part: Increased tumour incidences seen for groups exposed at 10 and 25 ppm for: **kidney adenocarcinoma** in male mice (0/54, 0/24, 3/21 (14.3%); **pulmonary adenoma** in male mice (3/80 (3.7%), 11/28 (39.3%), 7/28 (25%)) and female mice (4/92 (4.3%), 3/30 (10%), 7/29 (24.1%)); and **mammary tumours** (mainly carcinomas) in female mice (2/98 (2%), 6/30 (20%), 4/30 (13.3%)) (values presented in brackets correspond to results at 0, 10 and 25 ppm) Second part: Increases incidences of: **kidney adenocarcinoma** in male mice (0/66 vs 25/98 (25.5%)); **pulmonary adenoma** in male mice (3/74 (4%) vs 16/113 (14.2%)) and female mice (3/86 (3.5%) vs 11/118 (9.3%)); and **mammary tumours** (mainly carcinomas) in female mice (1/89 (0.1%) vs 12/118 (10.2%))	et al., 1977 Maltoni C. et al., 1984 Klimisch 3
No guideline followed GLP: not stated Albino CD-1 mice 36 mice/sex/dose	Vinylidene chloride Purity: 99% 0, 55 ppm (0, 220 mg/m^3) Whole body exposure 6 h/d, 5 d/week for 12 months Interim sacrifices at 1, 2, 3, 6 and 9 months	↗ incidence of **bronchioloalveolar adenoma** (1/26 controls vs 6/35 exposed) and no significant ↗ incidence of **haemangiosarcoma** of the liver (0/26 control vs 3/35 exposed) in males. 3 **hepatomas** [hepatocellular carcinomas] (2 in males, 1 in females) and 2 skin **keratoacanthomas** also reported in treated mice [sex unspecified]	Lee C.C. et al., 1977 Klimisch 3
Study on hamsters			
No guideline followed GLP: not stated Chinese hamsters 30 hamsters/sex/dose 17-18 hamsters/sex in control group	Vinylidene chloride Purity: 99.9% 0, 25 ppm (0, 100 mg/m^3) Whole body exposure 4 h/d, 4-5 days/week for 52 weeks	No significant increase of tumours	Maltoni C. et al., 1977 Maltoni C. et al., 1984 Klimisch 3

Table 18: Summary table of animal studies on carcinogenicity by oral route

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Studies in rats			

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Similar to OECD guideline 451 GLP: not stated Rats F344 50 rats/sex/dose	Vinylidene chloride Purity: 99% 0, 1, 5 mg/kg bw/d Exposure in corn oil by gavage Once a day, 5 d/week for 104 weeks	Survival and body weight similar in all treated and control groups. 12 control male rats and 10 male rats exposed to the low dose killed accidentally during week 82 of the study, and one male exposed to the low dose killed accidentally during week 42. No significant increase in tumour incidence observed in male and female rats treated with vinylidene chloride, when using life table analyses.	NTP, 1982 Klimisch 2
No guideline followed Similar to OECD guideline 451 GLP: not stated Rat SD 48 rats/sex/dose 80 rats/sex control group	Vinylidene chloride Purity > 99.5% 50, 100, 200 ppm (males: 7, 10, 20 mg/kg bw/d; females: 9, 14, 30 mg/kg bw/d) Exposure in drinking-water ad libitum for 2 years.	Mortality, mean organ weights, fasted body weights, organ to body weight ratios and body weight gain similar in the treated and control groups. Values for the hematological determinations and urinalysis of control and test groups within the normal range. No consistent or dose-related differences in clinical chemistry parameters in any of the test groups. In males, statistically significant ↗ incidence of hepatocellular fatty change and hepatocellular swelling in the 200 ppm group. A trend towards an ↗ incidence of hepatic changes observed in the 100 ppm group. No exposure-related hepatic changes in the 50 ppm group. Minimal hepatocellular fatty change and hepatocellular swelling in females at all dose levels. No significant hepatocellular necrosis in either male or female rats at any of the dose levels. No statistically significant increase in tumour incidence reported in treated rats	Quast et al., 1983 Klimisch 2
No guideline followed GLP: not stated SD Rats 50 rats/sex/dose 100 rats/sex control group Only the early results available; no data on statistical analyses	Vinylidene chloride Purity: 99.9% 0.5, 5, 10, or 20 mg/kg bw/d Exposure in olive oil by gavage once per day for 4–5 days per week for 52 weeks Observation for lifespan (up to 147 weeks)	Pattern and incidences of tumours comparable among treated and control rats.	Maltoni C. et al., 1977 Maltoni C. et al., 1984 Klimisch 3
No guideline followed GLP: not stated BD IV rats	Vinylidene chloride Purity: 99% Exposure in olive oil by gavage for 120 weeks (progeny) 24 females: single	Litter sizes, pre-weaning mortality, survival rates and body weight gain similar between the group treated with and the vehicle-control group. No statistically significant increase in the incidence of any tumours noted in exposed male or female offspring or in exposed dams.	Ponomarkov & Tomatis, 1980 Klimisch 3

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	dose of 150 mg/kg bw on day 17 of gestation Progeny (89 males and 90 females): 50 mg/kg bw once per week for life, beginning at weaning vehicle-control group: 14 dams, and their progeny (53 males and 53 females)		
Studies in mice			
Similar to OECD guideline 451 GLP: not stated B6C3F1/N mice 50 mice/sex/dose	Vinylidene chloride Purity: 99% 0, 2, 10 mg/kg bw/d Exposure in corn oil by gavage Once a day, 5 days per week for 104 weeks	No effect on survival in male and female mice. Mean body weights of the female mice given the high dose comparable with those of controls. Mean body weights of male mice given either dose and of female mice given the low dose slightly lower than those of controls. Significant increases in the incidence of tumours of the haematopoietic system in female mice given the low dose: malignant lymphoma (2/48; 9/49, P = 0.012 by life table test, and P = 0.028 by Fisher exact test; 6/50) and lymphoma or leukaemia (combined) (7/48; 15/49, P = 0.037 by life table test, and P = 0.050 by Fisher exact test; 7/50)*. Considered not relevant. *values presented in brackets correspond to results for control, 2 and 10 mg/kg bw/d	NTP, 1982 Klimisch 2

Table 19: Summary table of animal studies on carcinogenicity, other routes of exposure

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Subcutaneous study Female Ha:ICR Swiss mice	Vinylidene chloride Purity not provided Exposure: once per week for 548 days 2 mg: 30 mice Control: 100 mice	No local sarcomas were observed in the controls or treated mice.	Van Duuren et al., 1979 Klimisch 3
Cutaneous study Female Ha:ICR Swiss mice 30 mice/group Control: no treatment (n = 100) or treatment with acetone (n = 30).	Vinylidene chloride Purity not provided 40.0 or 121.0 mg Exposure: three times per week for 440–594 days	No skin papillomas were observed in the controls or mice treated with VDC.	Van Duuren et al., 1979 Klimisch 3

Table 20: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Initiation/promotion study Skin application Exposure: 3 times/week for 428–576 days	Vinylidene chloride Purity not provided	Female Ha:ICR Swiss mice Treated group: 30 mice, 121.0 mg + 14 day later 5 µg 12-O-tetradecanoylphorbol-13-acetate (TPA) TPA-treated: 90 mice, TPA only Positive controls: 30 mice, 20 µg 7,12-dimethylbenz[a]anthracene (DMBA) + TPA	In the vinylidene chloride + TPA group, 9 skin papillomas observed in 8 mice ($P < 0.005$ versus TPA controls); 1 mouse had a skin squamous cell carcinoma.	Van Duuren et al., 1979

10.7.1 Short summary and overall relevance of the provided information on carcinogenicity

Based on the data summarised in the table above and described in details in the following section, IARC assessed in 2017 the carcinogenicity of VDC (IARC, 2019). According to its own criteria, the IARC reached following conclusions:

- There is **inadequate evidence in humans** for the carcinogenicity of vinylidene chloride.
- There is **sufficient evidence in experimental animals** for the carcinogenicity of vinylidene chloride.

Vinylidene chloride is possibly carcinogenic to humans (Group 2B).

The most reliable and relevant carcinogenicity studies are those performed by the NTP (1982, 2015). Thus, they are considered by the DS as key studies for concluding on classification of VDC. For each experiment, the NTP assessed the statistical and biological significance of each tumors and the link between the different neoplasms observed and the exposure to VDC to conclude on the overall strength of evidence of carcinogenicity. For each neoplasm, the conclusions of the NTP are reported below and are endorsed by the DS, unless clearly specified.

The dataset is then discussed and assessed according to the CLP criteria in the subsequent section 10.7.2.

Oral route

In the framework of their technical report series, NTP assessed carcinogenic potency of vinylidene chloride in rats and mice by oral route (1982).

In the 2-year exposure study, 50 F344/N rats/sex and 50 B6C3F1/N mice/sex were exposed by gavage to VDC suspended in corn oil at dose levels of 0, 1 or 5 mg/kg bw/d and 0, 2 or 10 mg/kg bw/d respectively. The exposure to VDC had no effect on survival of mice and rats. However, while no significant differences in survival were observed for any group of rats, 12 control and 10 low-dose males were killed accidentally during week 82, and one during week 42; this may have compromised the sensitivity of the male rat study. Only the mean body weights of male mice given either dose and of female mice given the low dose were slightly lower than those of controls. The incidence of chronic inflammation of the kidney in both male and female rats was higher in high-dose animals than in controls. Although this lesion is common in aging rats, the occurrence appears to be dose related. In mice, necrosis of the liver (focal, multifocal or diffuse) was observed more frequently in dosed mice than in controls (male controls, 2%; low-dose 7%; and high-dose, 14%; female controls, 0%; low-dose, 8%; and high-dose, 2%). The only observed significant ($P < 0.05$) increase in tumour incidence occurred in low-dose female mice: **lymphoma** (2/48, 9/49, 6/50) and **lymphoma or leukemia combined** (7/48, 15/49, 7/50). These increases were considered not to be related to VDC administration because similar effects were not found in the high-dose female mice or in male mice or

rats. Therefore, NTP concluded that under the conditions of this bioassay, VDC administered by gavage was not carcinogenic. However, since the use of a maximum tolerated dose in this study has not been clearly demonstrated, and taking account the accidental deaths in rats, this study should not be taken as proof that the chemical is not a carcinogen. NTP (1982) and IARC (2019) shared these conclusions. DS concurres to the same conclusion.

Another study (Quast et al., 1983) exposed groups of 47–48 male and 48 female Sprague-Dawley rats (age, 6–7 weeks) to VDC in drinking-water for 2 years to equivalent concentrations of 7, 10, 20 mg/kg bw/d for males and 9, 14, 30 mg/kg bw/d for females. A group of 80 male and 80 female controls received drinking-water only. Mortality and body weight gain were similar in the treated and control groups, as values for the hematological determinations and urinalyses and clinical chemistry parameters. No statistically significant increase in tumour incidence was reported in treated rats. Even if some histopathological findings were observed (statistically significant increased incidence of hepatocellular fatty change and hepatocellular swelling in the 200 ppm group in males and minimal hepatocellular fatty change and hepatocellular swelling in females at all dose levels), the achievement of a maximum tolerated dose, at least for carcinogenic effects, is questionable. This would prevent the use of these results to conclude on carcinogenicity potential of VDC by oral route.

Other available studies did not report any increase in tumors incidence but are of limited quality, in particular methodology far from OECD guidelines, short duration of exposure, few details on protocol and/or results provided (Maltoni C. et al., 1977; 1984; Ponomarkov & Tomatis, 1980).

Overall, based on the available dataset, even if the studies did not report an increase of tumors, carcinogenicity by oral route cannot be excluded by DS.

Inhalation route

In the framework of their technical report series, NTP assessed carcinogenic potency of vinylidene chloride on rats and mice by inhalation route (2015):

- Rat study

Groups of 50 F344/N rats/sex (age, 5–6 weeks) were exposed by whole-body inhalation to VDC vapour at concentrations of 0 (control), 25, 50, or 100 ppm (0, 100, 200, 400 mg/m^3) for 6 hours per day, 5 days per week for 105 weeks. The survival of exposed groups of males was similar to that of controls. The survival of females exposed at 100 ppm was significantly less than that of controls (19/50 animals surviving to study termination against 30/50 in control group, P = 0.029). Mean body weights of exposed groups of male and female rats were similar to those of controls throughout the study.

In male, the incidences of **malignant mesothelioma** (mainly from the tunica vaginalis) occurred with a significant positive trend and were significantly increased in all exposed groups compared with the control group (1/50, 12/50, 28/50, 23/50). Importantly, these marked increases in the incidences of malignant mesothelioma in all exposed groups occurred with a concentration-dependent decrease in the time to first incidence (562, 535, 500 and 449 days). These malignant mesotheliomas are uncommon background in male F344/N rat (1/200; all routes: 26/699). A significant positive trend in the incidence of **adenoma of the nasal respiratory epithelium** was observed (0/49, 0/50, 1/50, 4/50); while the incidences observed are low, no nasal respiratory epithelium adenomas have been seen in NTP male historical controls, in any route of exposure. Importantly, nonneoplastic lesions also occurred in nose with statistically significant increased incidences and severities with increasing exposure concentration. These lesions included turbinate atrophy (0/49, 50/50, 50/50, 50/50) and hyperostosis (0/49, 49/50, 50/50, 50/50), respiratory metaplasia of olfactory epithelium (3/49, 49/50, 49/50, 48/50), chronic active inflammation (9/49, 36/50, 45/50, 48/50), respiratory epithelial hyperplasia (5/49, 8/50, 22/50, 31/50) and thrombosis (4/49, 4/50, 11/50, 7/50). These nonneoplastic lesions are consistent with chronic injury and repair, a process that has been linked with carcinogenesis and related to VDC exposure.**Renal tubule carcinomas** were observed in four males exposed (0/50, 2/50, 1/49, 1/50); these neoplasms are rare in male F344/N rats (NTP historical incidence: inhalation studies, 0/200; all routes, 1/697), and an increase in the incidence of renal tubule hyperplasia was also noted (3/50, 5/50, 6/49, 8/50), a lesion which can be considered precursor to neoplasm formation. These effects are related to VDC exposure.

In females, similarly to males, the incidence of **adenoma of the nasal respiratory epithelium** in animals exposed to the high concentration (1/50, 2%) also exceeded the NTP historical control range for inhalation studies (0/200; all routes, 1/697). As in males, nonneoplastic lesions also occurred in nose with statistically significant increased incidences and severities with increasing exposure concentration: turbinate atrophy (0/50, 50/50, 50/50, 50/50) and hyperostosis (0/50, 50/50, 50/50, 50/50), respiratory metaplasia of olfactory epithelium (1/50, 50/50, 50/50, 50/50), chronic active inflammation (7/50, 45/50, 46/50, 46/50), respiratory epithelial hyperplasia (4/50, 12/50, 14/50, 27/50), and thrombosis (0/50, 3/50, 2/50, 7/50). Rare **malignant mesotheliomas** (none have occurred in historical control group database) occurred in one female exposed to the low concentration (pleura and pericardium) and in one female exposed to the medium concentration (peritoneum). They are considered related to VDC exposure. Significantly increased incidences, with significant positive trends, were seen for **C-cell adenoma** of the thyroid gland in females exposed to the high concentration (3/50, 4/50, 6/48, 11/50) and significantly increased incidences were also observed for **C-cell carcinoma** of the thyroid gland in females exposed to the low concentration (0/50, 6/50, 2/48, 2/50). C-cell carcinoma are rare neoplasms in the F344/N rat and the incidences at the two highest concentrations, while not statistically significant, exceeded the historical control range for the inhalation route of exposure in female rats (1/200 (0.5% ± 1.0%); all routes: 6/690 (0.9% ± 2.0%)). Significantly increased incidence was seen for **C-cell adenoma or carcinoma (combined)** at the low and high concentrations (3/50, 10/50, 8/48, 13/50). However, as these lesions were not concentration related and were not accompanied by increased incidence of hyperplasia. Finally, the incidence of **mononuclear cell leukaemia** was significantly increased in the high concentration group, with a significant positive trend (10/50, 11/50, 13/50, 25/50). These increases in the incidences occurred with a concentration-dependent decrease in the time to first incidence (631, 451, 421 and 395 days). Mononuclear cell leukemia is a relatively common background neoplasm in F344/N rats, but the increase in the high dose group exceeded the historical control ranges for inhalation studies (20-34%) and all routes of administration (10-36%). Mice study

50 B6C3F1/N mice/sex (age, 5–6 weeks) were exposed by whole-body inhalation to VDC vapour at concentrations of 0 (control), 6.25, 12.5, or 25 ppm (0, 25, 50, 100 mg/m^3), for 6 hours per day, 5 days per week for 105 weeks. The survival of male mice exposed to the low concentration was significantly greater than that of controls; the survival of males exposed to the high concentration and the survival of females exposed to the low and high concentrations were significantly lower than that of the controls (animals surviving to study termination: males: 29/50, 40/50, 32/50, 19/50; females: 36/50, 25/50, 30/50, 24/50). Mean body weights of males exposed to the medium and high concentrations and females exposed to the high concentration were at least 10% lower at the end of the study (max -20%) than those of controls during the study. The NTP however did not consider these findings as modifying factor for the interpretation of the results.

In males, the incidences of **renal tubule adenoma** (0/50, 5/50, 19/50, 10/50)**, renal tubule carcinoma** (0/50, 7/50, 31/50, 18/50)**, and renal tubule adenoma or carcinoma (combined)** (0/50, 11/50, 37/50, 27/50) were significantly increased in all exposed groups, with a significant positive trend in the incidence of these tumours. Concomitantly, a concentration-dependent decrease in the time to first incidence is observed for adenoma in treated groups (729, 600 and 525 days). The incidences of renal tubule hyperplasia were also significantly increased in all exposed groups of males. No renal tubule hyperplasia, adenomas or carcinomas were observed in chamber control male mice or in 298 NTP historical control mice from inhalation studies. The incidences of **hepatocholangiocarcinoma** in exposed groups were non-significantly increased compared with that in the control group (1/50, 2/50, 2/50, 3/50) but exceeded the historical control range for inhalation studies: hepatocholangiocarcinoma has been reported in 2/299 (0.7%) inhalation controls and in 10/949 (1.1%) controls from all routes of exposure.

In females, the incidences of systemic **haemangioma** in all exposed groups were non-significantly increased when all organs where this lesion occurred were combined (0/50, 2/50, 2/50, 2/50) compared with controls. However, none were observed in the control group, or in any of the 300 NTP historical controls from inhalation studies. There was a significant positive trend (P = 0.044) in the incidence of systemic **haemangiosarcoma** (4/50, 4/50, 4/50, 9/50) when all organs where this lesion occurred were combined. These increases were predominantly driven by the statistically significant increase in the incidence of this neoplasm in the liver. Compared with controls, the incidence of systemic **haemangioma or haemangiosarcoma (combined)** (4/50, 6/50, 6/50, 11/50) in females exposed to the high concentration was

significantly greater, with a significant positive trend. The incidences of **hepatocellular adenoma** in females exposed to the medium concentration (25/50, 21/50, 36/50, 29/50), of **hepatocellular carcinoma** in females exposed to the high concentration (8/50, 14/50, 12/50, 17/50), and in **hepatocellular adenoma or carcinoma (combined)** in females exposed to medium and high concentrations (28/50, 30/50, 37/50, 38/50) were significantly greater than those in the control groups, with significant positive trends. These neoplasm are considered related to VDC exposure even if this is a common background in female B6C3F1 mice[1]. In addition, **hepatocholangiocarcinoma** occurred in all exposed groups of females (0/50, 1/50, 1/50, 2/50). In female B6C3F1 mice, this neoplasm is very rare and has not been observed in 300 inhalation controls or 948 controls from all routes of exposure in studies conducted by the National Toxicology Program (NTP). The incidence of **bronchioloalveolar carcinoma** was significantly increased in females exposed to medium concentration with a significant positive trend (1/50, 2/50, 7/50, 5/49). Also, time to first incidence was shorter in all exposed group compared to control group (731, 558, 392, 502). However, there was no increase in the incidences of alveolar/bronchiolar adenoma, no accompanying increase in incidence or severity of hyperplastic lesions, and no neoplastic effect in males. Incidences of alveolar/bronchiolar neoplasms were inside the NTP historical control data[2]. In females exposed to the high concentration, even if not statistically significant, the incidence of **carcinoma of the small intestine** (ileum) (3/50, 6%) exceeded the historical control ranges for inhalation studies (2/300) and all routes of administration (2/950) and may therefore have been related to treatment.

Based on the results of the two studies described above, the conclusions of NTP about the carcinogenic potential of VDC in rats and mice by inhalation is the following:

*"Under the conditions of this 2-year inhalation study, there was **clear evidence of carcinogenic activity** of vinylidene chloride in male F344/N rats based on increased incidences of malignant mesothelioma. Increased incidences of **renal tubule carcinoma** and **respiratory epithelium adenoma** in the nose of male rats were also considered to be related to vinylidene chloride exposure. There was **some evidence of carcinogenic activity** of vinylidene chloride in female F344/N rats based on increased incidences of **C-cell adenoma or carcinoma in the thyroid gland** and **systemic mononuclear cell leukemia**. Occurrences of malignant mesothelioma may have been related to vinylidene chloride exposure. There was **clear evidence of carcinogenic activity** of vinylidene chloride in male B6C3F1/N mice based on increased incidences of **renal tubule adenoma and carcinoma**. Increased incidences of **hepatocholangiocarcinoma** may have been related to vinylidene chloride exposure. There was **clear evidence of carcinogenic activity** of vinylidene chloride in female B6C3F1/N mice based on increased incidences of **systemic hemangioma or hemangiosarcoma** (combined)* (NTP, 2015)." Overall DS concurres to the same conclusions, except for leukemia in female rats. Indeed, regarding the positive trend, the statistically significant increase at the highest concentration and the higher incidence than in historical controls, DS considers that this can correspond to *sufficient evidence of carcinogenicity* according to CLP criteria (see also section 10.7.2).

Other data are available in the literature and are described below. DS considers these studies of low reliability and relevance considering the limitations listed in Table 17 above. They were therefore not used to conclude on classification since conclusions can be drawn based on well-conducted NTP studies.

Maltoni *et al.* (1984) performed 3 long term/carcinogenicity experiments by inhalation on rats, mice and hamsters. Protocols and results are described below.

[1] Hepatocellular adenoma : Historical incidence for inhalation studies: 105/300 (35.0% ± 8.8%), range 28%–50%; all routes: 378/948 (39.9% ± 18.7%), range 14%–78%.
Hepatocellular carcinoma: Historical incidence for inhalation studies: 44/300 (14.7% ± 5.0%), range 8%–20%; all routes: 152/948 (16.0% ± 10.6%), range 4%–46%.
Hepatocellular adenoma or Carcinoma: Historical incidence for inhalation studies: 133/300 (44.3% ± 8.6%), range 32%–56%; all routes: 448/948 (47.3% ± 19.3%), range 20%–82%.

[2] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 13/299 (4.4% ± 4.3%), range 0%–10%; all routes: 38/949 (4.0% ± 3.6%), range 0%–14%.

Four groups of 30 Swiss mice/sex were exposed to VDC at concentrations of 10, 25, 50 or 100 ppm (0, 40, 100, 200, 400 mg/m^3) and one group of 60 mice/sex was exposed to 200 ppm (800 mg/m^3) in air for 4 hours per day, 4–5 days per week, for 52 weeks and observed for their lifespan (up to 121 weeks). A group of 100 mice of each sex (age, 16 weeks) not kept in inhalation chambers served as one group of controls (control A). Concentrations of 200, 100 and 50 ppm caused high mortality and severe toxicity, causing termination of the assay at these concentrations. Compared with control A mice, increased tumour incidences were seen for groups exposed at 10 and 25 ppm for:

- **kidney adenocarcinoma in male mice** (0/54, 0/24, 3/21 (14.3%) [P = 0.02, Fisher exact]);
- **pulmonary adenoma in male mice** (3/80 (3.7%), 11/28 (39.3%; P < 0.05, Fisher exact), 7/28 (25%; P < 0.05, Fisher exact)) and **female mice** (4/92 (4.3%), 3/30 (10%), 7/29 (24.1%; P < 0.05, Fisher exact)); both of them occurred without dose response relationship and decrease in average latency of occurrence;
- **mammary tumours (mainly carcinomas) in female mice** without dose response relationship and decrease in average latency of occurrence (2/98 (2%), 6/30 (20%; P < 0.05, Fisher exact), 4/30 (13.3%; P < 0.05, Fisher exact)).

To increase the power of the study, additional groups of 120 Swiss mice of each sex (age, 9 weeks) were then exposed to VDC at a concentration of 25 ppm for their lifespan (up to 121 weeks) and observed concurrently with separate control groups of 90 mice of each sex (age, 9 weeks) not kept in inhalation chambers (control B). Comparisons of tumour incidences between control B mice and the groups of mice exposed concurrently at 25 ppm showed increases in the incidences of tumours at several sites: **kidney adenocarcinoma in male mice** (0/66 vs 25/98 (25.5%) [P < 0.0001, Fisher exact test]); **pulmonary adenoma in male mice** (3/74 (4%) vs 16/113 (14.2%); P < 0.05, Fisher exact test) and **female mice** (3/86 (3.5%) vs 11/118 (9.3%); P < 0.05, Fisher exact) and **mammary tumours (mainly carcinomas) in female mice** (1/89 (0.1%) vs 12/118 (10.2%); P < 0.05, Fisher exact). The authors concluded that **adenocarcinoma of kidney in mice is the only specific tumour linked to VDC exposure**, particularly since no corresponding tumors were noted in the control male mice. They also consider that the statistically significant increases of mammary carcinomas and pulmonary adenomas are difficult to evaluate since they were not dose-related and needs further clarification. Although the study is of low reliability (Klimisch score 3; see Table 17 above for details on limitations), the DS notes that the increase incidence of kidney neoplasms is in accordance with the observations made in the NTP studies in male rats and mice, and confirms the kidney as a target organ of carcinogenesis of VDC. DS also notes that increased mammary tumors are also reported by other authors (Quast et al. 1986; Cotti et al. 1988 - see below) in studies of limited reliability.

Thirty Sprague-Dawley rats/sex were exposed to VDC at 10, 25, 50, or 100 ppm (0, 40, 100, 200, 400 mg/m^3) for 4 hours per day, 4–5 days per week for 52 weeks, followed by observation for lifetime (up to 137 weeks). An additional group of 60 rats/sex was initially exposed at 200 ppm (800 mg/m^3) for 2 days, then 150 ppm (600 mg/m^3) for 4 hours per day, 4–5 days per week for 52 weeks, followed by observation for lifetime; the dosing frequency was reduced periodically to four times per week due to toxicity. Groups of 100 rats of each sex (age, 16 weeks) not kept in inhalation chambers were used as controls. An increase in the incidence of **mammary tumours** was observed in treated female rats when compared to the control group but without apparent dose-response trend. The pattern of neoplasms and their incidences were comparable among treated and control rats.

Groups of 30 Chinese hamsters/sex were exposed to VDC at 25 ppm (100 mg/m^3) in air for 4 hours per day, 4–5 days per week for 52 weeks, and observed for their lifetime (up to 164 weeks). A group of 18 males and 17 females, not housed in inhalation chambers, were used as controls. The pattern of neoplasms and their incidences were comparable among treated and control hamsters.

Because these studies suffer from several limitations (see table 17 above for more details), and particularly from a too short duration of exposure to study carcinogenic potential (52 weeks – Klimisch 3), they don’t allow for an adequate interpretation.

The team of Lee (Lee et al., 1977, 1978) undertook studies to determine the toxic and carcinogenic effects of VDC in rats and mice.

Groups of 36 CD-1 mice/sex (age, 2 months) were exposed to 0 (air, control group) or 55 ppm (220 mg/m^3) VDC in air for 6 hours per day, 5 days per week for up to 12 months, at which point the experiment was terminated. Four mice were killed at 1, 2, 3, 6, and 9 months from the start of the experiment. Statistically non significant increases in the incidence of **bronchioloalveolar adenoma** (1/26 controls vs 6/35 exposed) and **haemangiosarcoma** of the liver (0/26 control vs 2/35 exposed) were observed in males. Three **hepatomas** [hepatocellular carcinomas] (two in males and one in females) and two **skin keratoacanthomas** [a benign tumour of the follicular epithelium] were also reported to occur in treated mice [sex unspecified]. Such tumors have been reported to occur spontaneously in small numbers of mice at this age, even though they did not occur in control animals in this study.

Groups of 36 CD rats/sex (age, 2 months) were exposed to 0 (air, control group) or 55 ppm (220 mg/m^3) VDC in air for 6 hours per day, 5 days per week for up to 12 months (with interim terminations of 4 rats after 1, 2, 3, 6, and 9 months), at which time the experiment was terminated. Of the 36 exposed male rats, 2 developed haemangiosarcomas (not statistically significant), 1 in a mesenteric lymph node and 1 in the subcutaneous tissue. No haemangiosarcomas were observed in male controls. There was no treatment-related increase in tumour incidence in females.

Because these two experiments suffer from several limitations (see Table 17 above for more details), and particularly from the use of only one dose and a too short duration of exposure to study carcinogenic potential (12 months – Klimisch 3), they don't allow for an adequate interpretation.

Hong *et al.* (1981) also undertook studies on both mice and rats to investigate carcinogenic effects of VDC.

Groups of 8–12 male and 8–12 female CD-1 mice (age, 2 months) were exposed to VDC in air at 55 ppm (220 mg/m^3) for 6 hours per day, 5 days per week for 1, 3 or 6 months, and maintained without treatment for a further 12-month observation period. Unexposed control groups consisted of 16–28 mice of each sex. There was a decrease in survival in exposed males and females (46% mortality versus 20% in controls). The incidence of hepatocellular tumours was 10/60 (17%) in male controls and 4/28 (14%) in exposed males. **Bronchioloalveolar tumours** were observed in 8/60 (13%) male controls, 8/60 (13%) female controls, 4/28 (14%) exposed males, and 1/28 (3%) exposed females. One treated male had a **haemangiosarcoma of the mesentery**, a rare tumour.

Groups of male and female CD rats (age, 2 months) were exposed to 55 ppm (220 mg/m^3) VDC in air for 6 hours per day, 5 days per week, for 6 months (20 males and 20 females) or 10 months (14 males and 16 females). After treatment, all exposed groups were maintained without further exposure for 12 months, at which time the remaining rats were killed. Corresponding control groups of 20 and 16 rats (a total of 36 control rats per sex) were maintained on filtered air for the same treatment periods and then maintained for a further 12-month period. There was a decrease in survival in exposed males (with 79% mortality versus 38% in controls). A single **hepatic haemangiosarcoma** was observed in a male rat that had been exposed to VDC for 6 months. **Fibroadenoma** were noted in five females in the control group and five females exposed to VDC.

Because these two experiments suffer from several limitations (see Table 17 above for more details), and particularly the use of only one dose and a too short duration of exposure to study carcinogenic potential (10 months max. – Klimisch 3), they don't allow for an adequate interpretation.

Quast et al. (1986) exposed groups of 86 male and 86 female Sprague-Dawley rats (age, 6–7 weeks) to VDC at 0 (control), 10, or 40 ppm (0, 40, 160 mg/m^3) for 6 hours per day, 5 days per week for 1 month. Exposure was then increased to 25 or 75 ppm (100 and 300 mg/m^3) VDC for 17 months because of the lack of treatment-related effects at 10 and 40 ppm after 1 month of treatment. Surviving rats were held for an additional 6 months. There were no treatment-related effects on body weight gain or survival, except for a significant increase in mortality among females exposed at 75 ppm during months 14–24 of the study. Compared with controls, VDC caused a statistically significant increase in the incidence of **mammary gland**

adenocarcinoma in the females exposed at low concentrations (2/84; 7/86; 4/84). There was no significant increase in the incidence of any tumours in males. In its monograph, IARC (2019) noted some limitations, in particular the poor survival of females and the incorrect statistics of this study (Klimisch 3). As the mammary gland tumor only increased at the lowest dose and not at the highest tested dose, it doesn't allow for an adequate interpretation.

Finally, in its 2019 monograph, IARC cited a last study, but with many limitations (study design, only one dose, limited reporting…) (Cotti et al., 1988). In an exposure experiment *in utero*, groups of 60 or 54 pregnant female Sprague-Dawley breeder rats (age, 13 weeks) were exposed by whole-body inhalation to 0 (controls) or 100 ppm (400 mg/m^3) VDC for 4 hours per day, 5 days per week for 7 weeks, then for 7 hours per day, 5 days per week for 97 weeks, and then kept under observation until spontaneous death. Concurrently, groups of 62 male and 61 female offspring were exposed transplacentally beginning at day 12 of gestation, and by whole-body inhalation postnatally with the same regimen as the breeders described above. Along with 158 male and 149 female rats serving as unexposed controls, all were kept under observation until spontaneous death. Exposure to VDC did not affect survival, but caused a slight decrease in body weights in all exposed groups. In breeders, VDC caused statistically non-significant increases in the incidences of **benign and malignant tumours of the mammary gland and malignant tumours of the mammary gland**. Compared with controls, an increased incidence of **leukaemia** was found in exposed male (control, 12/158, 7.6%; exposed, 10/62, 16.1% [not statistically significant]) and female (control, 1/149, 0.7%; exposed, 4/61, 6.5% [$P < 0.03$]) offspring. Because this experiment suffer from several limitations (see Table 17 above for more details), it doesn't allow for an adequate interpretation.

Other routes of exposure

Van Duuren et al. (1979) investigated carcinogenic potential of VDC by subcutaneous injection or dermal application.

A group of 30 female Ha:ICR Swiss mice (age, 6–8 weeks) received subcutaneous injections of 2.0 mg VDC in 0.05 mL trioctanoin into the left flank once per week for 548 days [78 weeks]. A group of 30 mice received similar treatment with trioctanoin only (vehicle control). An additional group of 100 mice served as untreated controls. No local sarcomas were observed in the controls or treated mice.

Two groups of 30 female Ha:ICR Swiss mice (age, 6–8 weeks) were treated three times per week for 440–594 days with skin applications of 40.0 or 121.0 mg VDC in 0.2 mL acetone on the dorsal skin. Controls received no treatment (n = 100) or treatment with acetone only (n = 30). No skin papillomas were observed in the controls or treated mice.

These experiments suffer from several limitations, and particularly from the absence of information on systemic toxicity, the use of only one dose in the subcutaneous exposure experiments, and the few study details available (Klimisch 3).

The same team also tested VDC for its initiating activity in a two-stage mouse-skin assay (Van Duuren et al., 1979)

A group of 30 female Ha:ICR Swiss mice (age, 6–8 weeks) received a single skin application of 121.0 mg VDC in 0.2 mL acetone on the dorsal skin, followed 14 days later by applications of 5 µg 12-O-tetradecanoylphorbol-13-acetate (TPA) in 0.2 mL acetone 3 times per week for 428–576 days. Four other groups of mice received no treatment (n = 100), treatment with acetone only (n = 30), treatment with TPA only (n = 90), or treatment with 20 µg 7,12-dimethylbenz[a]anthracene (DMBA) plus TPA (n = 30), and served as untreated, vehicle, TPA-treated, or positive controls, respectively. Complete necropsies were performed at termination of the study or at death, and all abnormal-appearing tissues and organs were examined histologically. Routine sections of certain tissues and organs were examined with no further details. In the 90 TPA-only control mice, seven skin papillomas were observed in six mice; two mice had skin squamous cell carcinomas. In the VDC plus TPA group of 30 mice, nine skin papillomas were observed in eight mice ($P < 0.005$ versus TPA controls); one mouse had a skin squamous cell carcinoma. No skin papilloma or carcinoma was observed in the untreated or acetone controls. In the positive control group

(DMBA+TPA), 317 skin papillomas developed in 29 mice (P < 0.0005 vs TPA controls); 18 mice had skin squamous cell carcinomas. Regarding these results, VDC could be seen as a an initiating agent.

10.7.2 Comparison with the CLP criteria

According to the CLP Regulation, "*substances are allocated to one of two categories based on strength of evidence and additional considerations (weight of evidence)*".

- "*Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence*"

No data on human are available on vinylidene chloride, precluding a classification in category 1A.

- "*Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence*" […] *"Such evidence may be derived from animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen)."*

"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) <u>two or more species</u> of animals or (b) <u>two or more independent studies in one species</u> 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;"

Numerous studies are available in experimental animals. Various tumours occurred in different species and in both sexes. A classification in category 1B can therefore be considered.

Strength of evidence

"Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance."

By oral route, no carcinogenic effect was observed with VDC. However, there are doubts if a maximum tolerated dose was achieved in the main studies available (NTP, 1982). The quality of the other studies is not sufficient to conclude on the absence of carcinogenic effect by this route of exposure.

By inhalation route, many studies, of heterogeneous quality and with different protocols and species, assessed the carcinogenic effects of VDC. Among these studies, the 2 studies from NTP (2015) on rats and mice emerged as key studies due to their high quality (Klimisch 1). As detailed in the previous section, in these well-conducted studies, VDC exposure induced an increased incidence (compared to control group) of malignant neoplasms (and for some of them a combination of benign and malignant neoplasms) relevant for classification in both species, either males or females.

Neoplasms being statistically significant only when compared to control of the study are the following:

- malignant mesothelioma,
- renal tubule carcinomas, adenoma, or combined,
- C-cell adenoma, carcinoma or combined of the thyroid gland,
- mononuclear cell leukaemia,
- haemangioma or haemangiosarcoma,

hepatocellular adenoma or carcinoma.Based on the strength of evidence, the conditions for a classification in category 1B are then fulfilled.

Weight of evidence

As detailed in the CLP criteria for carcinogenicity (CLP 3.6.2.2.4), "*Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans*". Considering VDC, the main factors (species and strain; tumour type and background incidence; multi-site responses; progression of lesions to malignancy; reduced tumour latency; responses in single or both sexes; confounding effect by excessive toxicity; route of exposure) for each observed tumors (beyond the ones relevant based only on strength of evidence) are described and assessed in the table below. Concerning the mechanism of action and its relevance to human,, **VDC is metabolised into mutagen compounds (such as epoxides) and is proposed to be classified as a substance suspected of causing genetic defects (Muta 2)**, based on evidence for mutagenic activity in studies *in vitro* that include exogenous metabolic activation systems and in the available comet *in vivo*. There is no grounds to consider these metabolic pathways would not be relevant to humans. Moreover, the observation of tumors in liver and kidneys of mice and rats (and to a lesser extent in the lungs), as detailed above draw a coherent picture regarding the positive results in the liver and kidney in the Comet assay.

Beside these factors, another point, mentioned in CLP 3.6.2.2.6, which may be taken into account for the assessment of VDC carcinogenic potential is the structural similarity with analogous substance. In the present case vinyl chloride has a harmonised classification for carcinogenicity category 1A, H350. Both substances undertake a similar metabolic pathway, by being metabolized by CYP2E1 to electrophilic metabolites. Also, as highlighted by IARC (2019), "*tumour induction by vinylidene chloride in rodents shows many similarities to that of vinyl chloride, that is, both compounds induced tumours of the lung, tumours of the mammary gland, and hepatic haemangiosarcomas in mice. The induction of hepatic haemangiosarcomas in mice has also been observed with other vinyl halides (vinyl fluoride and vinyl bromide) that are metabolized by CYP2E1 to DNA-reactive haloethylene oxide intermediates. Hepatic haemangiosarcomas are extremely rare in the general population, but significantly elevated in workers exposed to vinyl chloride*"

Overall, the consideration of these additional factors confirms the level of concern for human carcinogenicity following the strength of evidence analysis. Classification as Carc. 1B is fully justified.

Table 21: Additional considerations for classification based NTP key studies (as part of a weight of evidence approach)

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
F344/N Rat	Malignant mesothelioma Males: uncommon background (inhalation route: 1/200; all routes: 26/699) Females: no cases in historical controls	Yes	Already malignant	Yes	Both	No	Inhalation	According to NTP (2015), inflammation is a well-known contributor to mesotheliomagenesis. Anti-inflammatory cytokines and chemokines were underrepresented in VDC-exposed mesotheliomas compared to spontaneous tumors, while pattern recognition receptors and damage-associated molecular pattern molecules were upregulated, consistent with immune dysregulation and a proinflammatory response. Responses such as these have been associated with mesothelial cell proliferation. The overrepresentation of these complex pathways supports the observation of a proinflammatory environment associated with mesotheliomas. These carcinogenic effects in animals are considered relevant to humans unless the opposite has been demonstrated.
	Adenoma of the nasal respiratory epithelium Males: no cases in historical controls Females: very uncommon background (inhalation route: 0/200; all routes, 1/697)		No	No	Both			The carcinogenic effects in animals are considered relevant to humans unless the opposite has been demonstrated.
	Renal tubule carcinomas Very uncommon background		Already malignant	NA	Single, males			See below for liver and kidney tumours These carcinogenic effects in animals are

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
	(inhalation route: 0/200; all routes: 1/697							considered relevant to humans unless the opposite has been demonstrated.
	Thyroid C-cell adenoma, carcinoma or combined Carcinoma: very uncommon background (inhalation route: 1/200; all routes: 6/690)		Yes	No	Single, females			These carcinogenic effects in animals are considered relevant to humans unless the opposite has been demonstrated.
	Mononuclear cell leukaemia Common background (inhalation route: 58/200; all routes: 165/700)		Already malignant	Yes	Single, females			These carcinogenic effects in animals are considered relevant to human unless the opposite has been demonstrated.
B6C3F1/N Mice	Renal tubule adenoma, carcinoma, or combined Very uncommon background (inhalation route: 0/298; all routes: 11/944)	Yes	Yes	Yes	Single, males	No		The mechanism by which VDC induces adverse effects in the liver and kidney may be related to the deactivation in the liver and reactivation in the kidney. VDC is metabolized in the liver by CYP2E1 to electrophilic metabolite VDC epoxide, and undergoes subsequent conjugation by glutathione or cysteine and is then transported to the kidney for excretion. In the kidney, cysteine-conjugated products become ideal substrates for β-lyase bioactivation to reactive metabolites. Carcinogenic effects in animals are considered relevant to human unless the opposite has been demonstrated.
	Hepatocholangiocarcinoma Males: very uncommon background (inhalation route: 2/299; all routes: 10/949) Females: no case in historical controls		Already malignant	No information	Both			
	Hepatocellular adenoma, carcinoma or combined Common background (inhalation route: 133/300; all routes: 448/948)		Yes	No	Single, females			
	Haemangioma, haemangiosarcoma or combined		Yes	No	Single, females			These carcinogenic effects in animals are considered relevant to human unless the opposite has been demonstrated.

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
	Uncommon background (inhalation route: 21/300; all routes: 55/950)							
	Alveolar/bronchiolar carcinoma Common background (inhalation route: 13/299; all routes: 38/949)		Already malignant	Yes	Single, females			These carcinogenic effects in animals are considered relevant to human unless the opposite has been demonstrated.
	Carcinoma of the small intestine Very uncommon background (inhalation route: 2/300; all routes: 2/950		Already malignant	No	Single, females			

10.7.3 Conclusion on classification and labelling for carcinogenicity

Regarding the data available, a classification as **Carc. Cat. 1B H350: May cause cancer** is warranted for VDC.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Even though there are several studies in the literature assessing the carcinogenic properties of VDC, the DS considered the carcinogenicity studies performed by the NTP (1982, 2015) as the most reliable and relevant studies and thus as the as key studies for concluding on classification. The NTP assessed the statistical and biological significance of each tumour type observed after VDC exposure, as well as the link between the different neoplasms and the exposure to VDC to conclude on the overall strength of evidence for carcinogenicity. NTP assessed carcinogenic potency of VDC in rats and mice by oral route (1982) and by inhalation route (2015).

In the oral studies the only observed significant increase in tumour incidence occurred in low-dose female mice: lymphoma and lymphoma or leukaemia combined (NTP, 1982). These incidences were considered not to be related to VDC administration because similar effects were not found in the high-dose female mice or in male mice or rats. On the other hand, several significant non-neoplastic lesions were observed. The incidence of chronic inflammation in the kidney in both male and female rats was higher in high-dose animals than in controls. Although this lesion is common in aging rats, the occurrence appeared to be dose related. In mice, necrosis of the liver was observed more frequently in dosed mice than in controls. Overall, NTP concluded that VDC administered by gavage was not carcinogenic. However, both NTP (1982) and IARC (2019) stated that this study should not be taken as a definitive proof that VDC is not a carcinogen because of the conditions used in the specific study.

As supporting evidence, the DS referred to Quast et al. (1983) who studied rats exposed to VDC in drinking-water for 2 years. No statistically significant increase in tumour incidence was reported, but the achievement of the maximum tolerated dose in order to be able to investigate carcinogenic effects was questionable according to the authors of the study. In addition, there were other available studies, however of limited quality, that did not report any increase in tumour incidence.

The DS shared the concerns raised by the NTP and IARC and considered that, even though the available studies by oral route did not report an increase in tumours, the high incidence and the severity of non-neoplastic lesions observed along with the low quality and almost 40-year-old studies available, carcinogenicity of VDC by oral exposure could not be excluded.

When carcinogenicity via the inhalation route was assessed in rats and mice in the NTP (2015) study, there was clear evidence of the carcinogenic activity of VDC in both species and sexes. In male rats increased incidences of malignant mesotheliomas were observed. Renal tubule carcinoma and respiratory epithelium adenoma in the nose of male rats were also considered to be related to VDC exposure. In female rats increased incidences of C-cell adenoma, carcinoma in the thyroid gland and systemic mononuclear cell leukaemia were reported, along with malignant mesothelioma. In male mice increased incidences of renal tubule adenoma and carcinoma and hepatocholangiocarcinoma were considered to be related to VDC exposure.

Similarly, in female mice increased incidences of systemic haemangioma or haemangiosarcoma (combined) were reported. Overall, the DS considered all the above-mentioned tumorigenic activity relevant for classification purposes, except leukaemia reported in female rats.

The DS also evaluated other studies of low reliability and relevance, as supporting evidence. Maltoni *et al*. (1984) concluded that adenocarcinoma of kidney in mice was the only specific tumour linked to VDC exposure (rats, mice and hamsters were tested), being consistent with the results of the NTP studies in male rats and mice. This result was considered to confirm that kidney was a target organ of carcinogenesis of VDC. Additionally, the DS could not interpret the relevance of the reported increased incidence of mammary tumours in Quast *et al*. (1986) and Cotti *et al*., (1988) and tumours reported by Lee *et al*. (1977, 1978) and Hong *et al*. (1981) due to limited reliability of the studies.

Finally, regarding dermal exposure, the DS discussed the Van Duuren *et al*. (1979) study which investigated the carcinogenic potential of VDC by subcutaneous injection or dermal application in mice. No skin papillomas or local sarcomas were observed in the controls or treated mice. These experiments suffered from several limitations (Klimisch 3). The same team also tested VDC for its initiating activity in a two-stage mouse-skin assay (Van Duuren *et al*., 1979). Regarding these results, VDC could be seen as an initiating agent.

Based on all the above, and along with the fact that VDC is metabolised into mutagenic compounds (such as epoxides) and was proposed to be classified as a substance suspected of causing genetic defects (Muta. 2), the DS believed that the level of concern for human carcinogenicity following the strength of evidence analysis was high, and therefore, classification as Carc. 1B was considered fully justified.

Comments received during consultation

One comment by an MSCA was received during the consultation supporting the proposed classification as Carc. 1B; H350.

Assessment and comparison with the classification criteria

Human evidence

The DS did not discuss any human evidence in the CLH report. Nevertheless, in the IARC assessment of VDC, a good-quality cohort study was mentioned investigating health effects among 4806 workers in a plastics manufacturing plant in the USA, who were exposed to vinyl chloride, polyvinyl chloride (PVC) dust, VDC, and several other chemicals (Waxweiler *et al*. 1981). A significant increase in mortality due to lung cancer was observed but no association between lung cancer and exposure to VDC was reported. Two smaller occupational cohort studies (Ott *et al*., 1976; Thiess *et al*., 1979) with co-exposure to vinyl chloride monomer and acrylonitrile had significant limitations and were considered of limited value.

Animal studies

RAC considered all studies summarised by the DS in Tables 17-20 of the CLH report for the evaluation of the carcinogenic activity of VDC. Thirteen studies were performed in rats (8 via inhalation route, 5 via oral route), 7 in mice (5 via inhalation route, 1 via oral route, 1 via dermal route) and 2 inhalation studies on hamsters. Inhalation (whole body exposure) studies by NTP 2015 (Klimisch score 1) in rats and mice and those by Rampy *et al.* (1977), Quast *et*

al. (1986), along with the oral (by gavage) NTP 1982 study and the oral study by Quast *et al.* (1983) study (in drinking water at libidum, Klimisch score 2) are considered of high reliability by RAC. The other studies were evaluated by the DS as Klimisch score 3. The carcinogenicity study via dermal exposure by Van Duuren *et al.* (1979) examined mice exposed to VDC cutaneously and sub-cutaneously. They also performed an initiation/promotion study using tetradecanoylphorbol-13-acetate as a pre-treatment agent, but the study was considered by DS as Klimisch score 3, mainly due to limited reporting details. In the following table a summary of the observed incidence of neoplastic (benign and malignant tumours) and non-neoplastic lesions relevant for VDC classification purposes as a carcinogen, is presented, as retrieved from all the studies evaluated in the CLH report.

Table: *Summary of the reported neoplastic (benign and malignant tumours) and non-neoplastic lesions relevant for the assessment of the carcinogenic properties of VDC*

Study	**Target organ**	**Lowest dose tumours/lesions are observed**	**Neoplastic and non-neoplastic incidences**	**Mortality and body weight changes compared to controls**
Oral route				
NTP, 1982 (Klimisch 2) Rats	Liver Kidney Pituitary Adrenals Pancreas Haematopoietic system	1 mg/kg bw/d	<u>Primary tumours</u> (most of them statistically significant by the Fisher exact test or by the Cochran-Armitage linear trend test, NOT significant when life table analyses used) Males Subcutaneous tissue – fibromas Haematopoietic system – leukaemia Pituitary – adenomas Adrenal – Pheochromocytomas Pancreatic islets – Islet-cell adenomas and carcinomas Testis – Interstitial-cell Tumours Females Haematopoietic system – leukaemia, lymphomas Liver - Neoplastic nodule Pituitary – adenomas Adrenal – Pheochromocytomas <u>Non-neoplastic lesions</u> Chronic renal inflammation in both	Survival rates and body weight gains similar to controls In male rats 12 controls and 10 low-dose animals were accidentally killed during week 82 of the study NOAEL 5 mg/kg bw/day, the highest exposure tested for carcinogenicity

			sexes (possible age-related)	
NTP, 1982 (Klimisch 2) Mice	Haematopoietic system Liver	2 mg/kg bw/d	Female Haematopoietic system – leukaemia, lymphomas, malignant lymphomas <u>Non-neoplastic findings</u> Necrosis of the liver (focal, multifocal or diffuse) in high-dosed males and low-dosed females	Survival rates similar to controls Body weight gain in males similar to controls, in females 9.7% decrease at 2 mg/kg bw/day, 4.1% decrease at 10 mg/kg bw/day NOAEL 10 mg/kg-day, the highest exposure tested for carcinogenicity
Quast et al., 1983 (Klimisch 2) Rats	Liver	Males 20 mg/kg bw/d Females 9 mg/kg bw/d	Only non-neoplastic lesions in the liver Midzonal fatty change in females and males only at the 20 mg/kg bw/day	The mortality among the test animals was comparable to the controls Mean body weights of the rats over the 2-year period were similar for all groups
Ponomarkov & Tomatis, 1980 (Klimisch 3) Rats	Rare scattered incidences stomach liver rectal salivary gland	Pregnant females: 150 mg/kg bw once Males/Females Progeny: 50 mg/kg bw 1/week	Pregnant females Hyperplastic liver nodules (2/23) Male progeny 1 squamous-cell carcinoma of the stomach 1 liver carcinoma 1 seminoma 1 rectal adenomatous polyp. Hyperplastic liver nodules 2/81 Meningiomas increased non-statistically significantly Female progeny, 2 liver cell carcinomas, and 1 liver cell adenoma	Survival rates and body weight similar to controls

			1 carcinoma and 1 adenoma of the salivary gland Hyperplastic liver nodules 6/80	
Inhalation route				
NTP, 2015 (Klimisch 1) Rats	Nose Kidney, Thyroid gland, Internal organs (mainly epididymis and testes), Liver	100 mg/m³	Males Malignant mesothelioma (mainly from the tunica vaginalis) Adenoma of the nasal respiratory epithelium Renal tubule carcinomas Females Malignant mesotheliomas (1 at 100 mg/m³, 1 at 200 mg/m³) Adenoma of the nasal respiratory epithelium. C-cell adenomas/carcinomas of the thyroid gland Mononuclear cell leukaemia	<u>Survival</u> • 100 mg/m³ Males 28/50 vs control 26/50 Females 27/50 vs control 34/50 • 400 mg/m³ Males 21/50 vs control 26/50 Females 21/50 vs control 34/50 <u>Body weight gain</u> • 100 mg/m³ Males Difference vs control – 0.2% Females Difference vs control + 4.5% • 400 mg/m³ Males Difference vs control – 3.8% Females Difference vs control + 0.8%
NTP, 2015 (Klimisch 1) Mice	Pulmonary system Nose Kidney Liver, Vascular system Small intestine Uterus	25 mg/m³	Males: Renal tubule adenoma, renal tubule carcinoma, renal tubule hyperplasia hepato-cholangiocarcinoma Females: Haemangioma and haemangiosarcoma of the vascular system Liver haemangiosarcoma Hepatocellular adenoma, carcinoma Hepato-cholangiocarcinoma Bronchioloalveolar carcinoma Carcinoma of the small intestine (ileum)	<u>Survival</u> • 25 mg/m³ Males 40/50 vs control 30/50 Females 26/50 vs control 37/50 • 100 mg/m³ Males 19/50 vs control 30/50 Females 26/50 vs control 37/50 <u>Body weight gain</u> • 25 mg/m³ Males Difference vs control – 3.5% Females Difference vs

				control – 13.7% • 100 mg/m³ Males Difference vs control – 25.4% Females Difference vs control – 51%
Maltoni et al., 1977 Maltoni et al., 1984 (Klimisch 3) Rats	Mammary gland	600 mg/m³	Females Mammary tumours	<u>Survival</u> Females 59/100 vs 98/10 in the control group No data on body weight gain
Maltoni et al., 1977 Maltoni et al., 1984 (Klimisch 3) Mice	Kidney, Mammary gland Pulmonary system	40 mg/m³	Kidney adenocarcinomas Mammary tumours Pulmonary adenomas	100 mg/m^3 was found to be the highest tolerable dose by mice
Lee et al., 1977 (Klimisch 3) Rats	(liver)	220 mg/m³	Non neoplastic lesions Liver: mild to markedly severe focal, disseminated vacuolisation, probably fatty change Neoplastic Haemangiosarcoma in the mesenteric lymph node or subcutaneous tissue 2/36 animals	No remarkable adverse signs One female rat terminated No deaths in the control group. Body weights of the female rats were generally less than that of the female controls after the 4th week. Body weights of the males generally less than that of the male controls after the 24th week.
Lee et al., 1977 (Klimisch 3) Mice	Pulmonary system Liver	220 mg/m³	Bronchioloalveolar adenoma Haemangiosarcoma of the liver in males. 3 hepatomas [hepatocellular carcinomas] 2 skin keratoacanthomas	Two males were terminated during the 9th month and one female during the 10th month (all liver tumours). Weight gains of the male and female mice

				comparable to those of the controls
Rampy et al., 1977 / Quast et al., 1986 (Klimisch 3) Rats	Mammary gland	100 mg/m³	Females Mammary gland adenocarcinoma	Mortality was slightly higher in the 100 mg/m³ group in the very last months of the study. For female rats at 100 mg/m³, a trend towards increased cumulative percentage of mortality was noted during the 14th to the 24th months (statistically significant at months 15, 17 and 21). Mean body weights of male rats at 100 mg/m³ significantly lower than the controls for the first 13 months of exposure and remained lower. Mean body weights of male rats at 300 mg/m³ were significantly lower from 6th to 12th months. General trend toward decreased body weights from the 17th to 24th months. Mean body weights of female rats comparable to controls or slightly higher.
Cotti et al., 1988	Haematopoietic system	400 mg/m³	Benign and malignant tumours of the	Slight decrease in

	Mammary gland		mammary gland Leukaemia	body weight in males and females
Dermal				
Van Duuren et al., 1979 (Klimisch 3) Mice			No local sarcomas, no skin papillomas When tested as initiating agent with PMA as promoter, 9 skin papillomas in 8 mice, 1 skin squamous cell carcinoma in 1 mouse	No data available

Mouse is the most sensitive species and male is the most sensitive sex for certain types of toxicity by VDC. Various studies ranging from acute to chronic toxicity confirm this observation in accordance with the metabolic pathway presented in the toxicokinetics section. However, this is not clearly established for carcinogenicity as different tumours at different doses with different latencies are observed in the different species tested.

By the oral route, the most reliable NTP (1982) study does not reveal a clear carcinogenic effect in rats and mice. However, there are several primary tumours reported in rats but the accidental deaths that occurred during week 82 of the study have an impact on statistics. Most of these tumours are statistically significant by the Fisher exact test or by the Cochran-Armitage linear trend test but become not significant when life table analyses are used. In addition, there is doubt as to whether the maximum tolerated dose (MTD) was achieved, since mortality rates and body weights of controls and dosed groups are similar. Other available studies report either only non-neoplastic lesions (Quast *et al*., 1983) or scattered incidences of tumours, but are of limited quality, in particular since the methodology followed is significantly different from the OECD TGs, short duration of exposure is applied and few details on the protocol and/or results are provided (Ponomarkov & Tomatis, 1980). Therefore, RAC considers that the available evidence is not sufficient to conclude on the absence of carcinogenic effect by the oral route of exposure.

By the inhalation route, the well-conducted NTP studies (2015) in mice and rats are relevant for the assessment of carcinogenicity. In the following tables, the incidences of tumours (both malignant and benign) per tested dose are summarised. Relevant tumours were found in both sexes in rats and mice in the absence of excessive toxicity. The incidence of malignant neoplasms and/or the combination of benign and malignant neoplasms was increased and statistically significant compared to controls in the following tumors: malignant mesothelioma, renal tubule carcinomas, adenoma, or combined, C-cell adenoma, carcinoma or combined of the thyroid gland, mononuclear cell leukaemia, haemangioma or haemangiosarcoma, hepatocellular adenoma or carcinoma. Moreover, some tumours showed reduced tumour latency, as indicated in table 21 of the CLH report. Therefore, the carcinogenic activity of VDC via inhalation can be concluded.

Table: *Tumours (malignant and benign) incidences (statistical significance* ***P<0.05 in bold****) in the NTP (2015) inhalation rat study relevant for classification*

Type of tumour	**Sex**	**Dose (mg/m³)**			
		0	**100**	**200**	**400**
Rats					
Malignant mesothelioma	Male	1/50	**12/50**	**28/50**	**23/50**
	Female	0/50	1/50	1/50	0/50
Thyroid gland adenomas/ carcinomas	Female	3/50	**10/50** (**6 carcinomas**)	9/48/	**13/50** (2 carcinomas)
Mononuclear cell leukaemia	Female	10/50	11/50	13/50	**25/50**
Renal tubule adenoma/ carcinoma	Male	3/50	4/50 (2 carcinomas)	6/49 (1 carcinoma)	2/50 (1 carcinoma)
	Female	0/50	0/50	1/49 (1 carcinoma)	0/50
Clitoral gland adenoma/ carcinoma	Female	5/47 (1 carcinoma)	8/48	3/45	9/48 (5 carcinomas)
Respiratory epithelium adenoma (nose)	Male	0/49	0/50	1/50	**4/50** (P=0.051)
	Female	0/50	0/50	0/50	1/50

Table: *Tumours (malignant and benign) incidences (statistical significance* ***P<0.05 in bold****) in the NTP (2015) inhalation mice study relevant for classification*

Type of tumour	**Sex**	**Dose (mg/m³)**			
		0	**25**	**50**	**100**
Mice					
Hepatocellular adenoma	Male	37/50	35/50	33/50	25/50
	Female	37/50	30/50	30/50	29/50
Hepatocellular carcinoma	Male	26/50	19/50	15/50	29/50
	Female	9/50	16/50	14/50	**20/50**
Hepatocholangioma	Male	1/50	2/50	2/50	3/50
	Female	0/50	1/50	1/50	2/50
Renal tubule adenoma/ carcinoma	Male	0/50	**11/50** (6 carcinomas)	**37/49 (24 carcinomas)**	**27/50 (18 carcinomas)**
	Female	0/50	0/50	0/50	1/50
Haemangioma/ Haemangiosarcoma	Female	4/50	6/50	6/50	**11/50**
Alveolar/ bronchial adenoma/	Female	4/50 (1	5/50 (2	9/50 (7	7/49 (5

carcinoma		carcinoma)	carcinomas)	carcinomas)	carcinomas)
Small intestine adenoma/ carcinoma	Male	1/50	3/50	1/50	2/50
	Female	2/50	1/50	2/50	4/50

Other studies, as shown in the table "Summary of the reported neoplastic (benign and malignant tumours) and non-neoplastic lesions relevant for the assessment of the carcinogenic properties of VDC" above, report mammary tumours, kidney adenocarcinomas, pulmonary and bronchioloalveolar adenomas, both in male and female rats and mice but can only be used as supporting evidence, as these studies suffer from several limitations. For example, studies were conducted with too short duration of exposure to study the carcinogenic potential (e.g., 52 weeks or less), or with only one dose applied or with not enough animals and in addition the results were inconsistent and/or unreliable as the tumourigenic response may have appeared only in the low dose, with no dose-related increase in tumours compared to controls etc. Nevertheless, the increase, for example, in the incidence of kidney neoplasms reported by Maltoni *et al.* (1984) is consistent with the observations made in the NTP (2015) studies in male rats and mice and increases the confidence that the kidney is a target organ of carcinogenesis of VDC. In addition, mammary tumors were also observed in other supporting studies (Maltoni *et al*., 1984; Quast *et al*., 1986; Cotti *et al*., 1988).

Via dermal exposure, data is limited and suffers from serious limitations, especially from the absence of information on systemic toxicity, the use of only one dose and the few study details available. No neoplasms were reported, but when tested for its initiating activity in a two-stage mouse-skin assay, VDC could be regarded as an initiating agent, as skin papillomas and skin squamous cell carcinomas were developed in mice (Van Duuren *et al*., 1979). Therefore, the carcinogenic activity of VDC via dermal exposure cannot be excluded.

In conclusion, there are several lines of evidence pointing to the carcinogenic activity of VDC. More specifically:

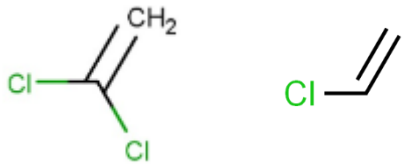
1. There is sufficient evidence of carcinogenic activity of VDC in male F344/N rats based on increased incidences of malignant mesotheliomas. There is also clear evidence of carcinogenic activity of VDC based on increased incidences of renal tubule adenoma and carcinoma in male B6C3F1/N mice and systemic haemangioma or haemangiosarcoma (combined) in female B6C3F1/N mice. Supporting evidence is derived from studies showing various tumors as already explained above and shown in the preceding table, hence VDC is considered as a multisite carcinogen in both sexes of mice and rats.

2. The sufficient evidence demonstrating the carcinogenicity of VDC in experimental animals, as explained above, originates from studies using the inhalation route of exposure. However, due to insufficient and/or low-quality data, the carcinogenic effect of VDC via oral or dermal exposure cannot be ruled out, and the route of exposure will not be stated in the hazard statement.

3. RAC considers that VDC also warrants classification as a suspected mutagen, based on evidence for mutagenic activity in *in vitro* studies that include exogenous metabolic

activation and in the available *in vivo* comet assay. The observation of tumors in liver and kidneys of mice and rats (and to a lesser extent in the lungs), are fully consistent with the positive results in the same tissue of the comet assay and suggest a mutagenic MoA for the tumours. VDC is metabolised to mutagenic compounds (e.g., epoxides) and there is no evidence that this (or any other) potential mechanism of carcinogenicity is not relevant to humans.

4. VDC possesses a high degree of structural similarity with vinyl chloride (see the Figure below), which has a harmonised classification (Index number 602-023-00-7) for carcinogenicity in Category 1A, H350. According to the CLP Regulation, 3.6.2.2.6, structural similarity to substance(s) for which there is good evidence of carcinogenicity may be taken into consideration when assessing the overall level of concern. Both substances undertake a similar metabolic pathway, by being metabolised by CYP2E1 to electrophilic metabolites. In the IARC assessment of VDC (IARC, 2019) it is noted that tumour induction by VDC in rodents shows many similarities to that of vinyl chloride: both compounds induced tumours in the lung, tumours of the mammary gland, and hepatic haemangiosarcomas in mice. The induction of hepatic haemangiosarcomas induced in mice has also been observed with other vinyl halides (vinyl fluoride and vinyl bromide) that are metabolised by CYP2E1 to DNA-reactive haloethylene oxide intermediates. Hepatic haemangiosarcomas are extremely rare in the general population, but significantly elevated in workers exposed to vinyl chloride.



Vinylidene chloride | Vinyl chloride

Figure: Structural similarity of vinylidene chloride and vinyl chloride

Therefore, taking into consideration all the above, RAC agrees with the DS that a **classification as Carc. 1B; H350: May cause cancer is warranted for VDC.**

10.8 Reproductive toxicity

Not assessed in this dossier.

10.9 Specific target organ toxicity-single exposure

Not assessed in this dossier.

10.10 Specific target organ toxicity-repeated exposure

Table 22: Summary table of animal studies on STOT RE by oral route

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Studies on rats			
Exposure in drinking-water ad libitum for 2 years. GLP: not stated Rat SD 48 rats/sex/dose 80 rats/sex control group	Vinylidene chloride Purity > 99.5% 50, 100, 200 ppm in drinking water for 2 years (males: 7, 10, 20 mg/kg bw/d; females: 9, 14, 30 mg/kg bw/d)	Hepatic changes, when present, were usually characterized by minimal amount of mid-zonal hepatocellular fatty change in both male and female rats. No significant hepatocellular necrosis in either male or female rats at any of the dose levels. In males, statistically significant ↗ incidence of hepatocellular fatty change and hepatocellular swelling in the 200 ppm group. A trend towards an ↗ incidence of hepatic changes observed in the 100 ppm group. No exposure-related hepatic changes in the 50 ppm group. In females, hepatocellular fatty change and hepatocellular swelling at all dose levels. No treatment-related effect on mortality, body weight, organ weight, hematology, urinalysis, biochemistry. NOAEL : 200 ppm = 20-30 mg/kg bw/day (only minimal changes) **No classification** (2-year GV (guidance value) range for STOT RE: ≤ 12.5 mg/kg bw/day)	Quast et al., 1983 Klimisch 2
Similar to OECD guideline 451 Rats F344 50 rats/sex/dose GLP: not stated Haematological examination, urinalysis, clinical chemistry not performed	Vinylidene chloride Purity: 99% 0, 1, 5 mg bw /kg/d Exposure in corn oil by gavage Once a day, 5 days per week for 104 weeks	Body weight similar in all treated and control groups. While no significant differences in survival observed for any group of rats, 12 control and 10 low-dose males killed accidentally during week 82. Incidence of **chronic inflammation of the kidney** in both male and female rats higher in high-dose animals than in controls (males: controls = 26/50, 52%; low-dose = 24/48, 50%; high-dose = 43/48, 90%; females: controls = 3/49, 6%; low dose = 6/49, 12%; and high-dose = 9/44, 20%). LOAEL: 5 mg/kg bw/d NOAEL: 1 mg/kg bw/d **Category 2** (2-year GV range for STOT RE 2: 1.25-12.5 mg/kg bw/day)	NTP, 1982 Klimisch 2

Subchronic study Rats F344 10 rats/sex/dose GLP: not stated No data on ophtalmological and haematological examination available.	Vinylidene chloride Purity: 99% 0, 5, 15, 40, 100, 250 mg/kg bw/d Exposure in corn oil by gavage Once a day, 5 days per week for 13 weeks	3 females receiving 250 mg/kg bw/d died during the first week. Weight gain depressed 20% for male rats receiving 250 mg/kg bw/d compared with controls. 250 mg/kg bw/d: Severe centrilobular necrosis of the liver in the 3 females that died. Minimal to moderate hepatocytomegaly in the rest of the rats. Various combinations of portal and subcapsular fibrosis, bile duct hyperplasia, pigmented macrophages, and hepatocellular atrophy in all males (mild to severe in 9/10 and minimal in 1/10) and in 7/10 females (mild to moderate in 6/10 and minimal in 1/10). Foci of cytoplasmic change, primarily clear cell foci, in 3/10 males and 3/10 females. 100 mg/kg bw/d: Lesser degrees (minimal to mild) of hepatocytomegaly in 6/10 males and 3/10 females. **Portal and subcapsular fibrosis, bile duct hyperplasia, pigmented macrophages, and hepatocellular atrophy**: rats affected to a much lesser degree, both in numbers and in severity compared to 250 mg/kg bw/d group. Fatty metamorphosis or cytoplasmic vacuolization or both, usually in minimal or mild degrees of severity, occurred in the animals of most groups but had no distinct dose relationship. LOAEL: 100 mg/kg bw/d NOAEL: 40 mg/kg bw/d **Category 2** (90-day GV range for STOT RE 2: 10-100 mg/kg bw/day)	NTP, 1982 Klimisch 2
Studies on mice			
Similar to OECD guideline 451 B6C3F1/N mice 50 mice/sex/dose GLP: not stated Haematological examination, urinalysis, clinical chemistry not performed	Vinylidene chloride Purity: 99% 0, 2, 10 mg bw /kg/d Exposure in corn oil by gavage Once a day, 5 days per week for 104 weeks	No effect on survival in males and females and on mean body weights of females in the high dose group. Mean body weights of males given either dose and of females given the low dose slightly lower than those of controls. **Necrosis of the liver** (focal, multifocal, or diffuse) more frequent in dosed mice than in controls (male controls, 1/46, 2%; low-dose 3/46, 7%; high-dose, 7/49, 14%; female controls, 0/47, 0%; low-dose, 4/49, 8%; high-dose, 1/49, 2%). LOAEL: 10 mg/kg bw/d NOAEL: 2 mg/kg bw/d **Category 2** (2-year GV range for STOT RE 2: 1.25-12.5 mg/kg bw/day)	NTP, 1982 Klimisch 2

Subchronic study similar to OECD guideline 408 B6C3F1/N mice 10 mice/sex/dose GLP: not stated No data on ophtalmological and haematological examination available.	Vinylidene chloride Purity: 99% 0, 5, 15, 40, 100, 250 mg /kg bw /d Exposure in corn oil by gavage Once a day, 5 days per week for 13 weeks	All males receiving 250 mg/kg bw/d died within 24 hours; 9/10 females receiving 250 mg/kg bw/d died within 48 hours. Deaths occurred in 1/10 females receiving 5 mg/kg bw/d; 1/10 females receiving 15 mg/kg bw/d; 1/10 males receiving 40 mg/kg bw/d; and 2/10 males and 3/10 females receiving 100 mg/kg bw/d. Dose-related decrease in mean body weight gain for male mice. Centrilobular necrosis, hemorrhage and congestion of the liver were observed in the males and females that died in the 250 mg/kg bw/d dose group. **Cellular atypia of the liver** (less severe than in the rats) in 7/10 males and 6/10 females receiving 100 mg/kg bw/d but not in animals receiving 250 mg/kg bw/d. Incidence of hepatic lesions in males dose related and higher than that in females. The most frequently encountered change in mice exposed to 40 mg/kg bw/d or less was slight, sometimes moderate, fatty metamorphosis (2 males and 2 females at 40 mg/kg bw/d). Patchy foci of one or a few smaller cells with sparse cytoplasm encountered much less frequently in mice than in rats. LOAEL: 100 mg/kg bw/d NOAEL: 40 mg/kg bw/d **Category 2** (90-day GV range for STOT RE 2: 10-100 mg/kg bw/day)	NTP, 1982 Klimisch 2
Study on dogs			
Subchronic study Beagle dogs 4 dogs/sex/dose GLP: not stated	Vinylidene chloride Purity > 99.5% 6.25, 12.5, 25 mg/kg bw/d Exposure in gelatine capsules VDC in peanut oil. Each day for 97 days	No exposure related changes in appearance and demeanor, body weights or food consumption. ↘ in mean white blood cell counts at 6.25 and 25 mg/kg bw/d, and changes in serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase and blood urea nitrogen at 6.25 mg/kg bw/d. All values within the range of normal values observed in this laboratory. No exposure related changes observed in any of the parameters examined on urinalysis, in organ weights or organ to body weight ratios or in gross and microscopic examination of the tissues from either the male or female dogs at any dose level. Pathologic changes interpreted to be spontaneous in occurrence and comparable in control and test dogs. LOAEL: / NOAEL: 25 mg/kg bw/d **No classification** (90-day GV range for STOT RE: ≤ 100 mg/kg bw/day)	Quast et al., 1983 Klimisch 2

Effects in bold are those considered for classification purpose

Table 23: Summary table of animal studies on STOT RE by inhalation

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**

Studies in rats			
Subacute study GLP compliant Whole body inhalation F344 rats 5 rats/sex/dose	Vinylidene chloride Purity > 99% 0, 25, 50, 100, 200, 400 ppm (0, 100, 200, 400, 800, 1600 mg/m^3) 6 hours/day, 5 days/week for two weeks	All male and 9/10 female rats in the 200 and 400 ppm groups found dead by day 2; one 400 ppm female found dead on day 4. All other rats survived the entire study except one 25 ppm male removed from the study due to non exposure-related condition. Mean body weight gain of 100 ppm females significantly less than that of the chamber controls. Final mean body weights of male and female rats exposed to 100 ppm 3% and 6% less, respectively, than those of the chamber control groups. Absolute and relative kidney weights of surviving groups of exposed males and females significantly greater than those of the chamber controls. In males, relative lung weights increased at 100 ppm compared to controls, and increasing trend observed in absolute and relative lung weights. Liver: centrilobular necrosis associated with early deaths in male and female rats exposed to 200 or 400 ppm VDC. **Centrilobular cytoplasmic alteration** of hepatocytes in all exposed groups of male and female rats surviving at terminal kill. Hepatocytic centrilobular cytoplasmic alteration characterized by decreased cytoplasmic staining, perinuclear halos, and flocculent cytoplasm. Mean severity of this alteration slightly higher in males. Centrilobular cytoplasmic alteration likely represents a form of hepatocellular degeneration, as rats exposed to 200 and 400 ppm did not have cytoplasmic alteration but rather centrilobular necrosis, consistent with a more severe stage of hepatocellular damage. **Renal tubule casts** in the renal papillae of 200 and 400 ppm rats, characterized by the presence of variable amounts of finely granular, brightly eosinophilic material in dilated tubule lumens of the renal papillae. LOAEC: 100 mg/m^3 (0.1 mg/L) NOAEC: / **Category 1** (14-day GV range for STOT RE1 (vapour): ≤ 1.2 mg/L/6h/day)	NTP, 2015 Klimisch 1
Subchronic study similar to OECD guideline 413 GLP compliant Whole body inhalation F344 rats 10 rats/sex/dose	Vinylidene chloride Purity > 99% 0, 6.25, 12.5, 25, 50, 100 ppm (0, 25, 50, 100, 200, 400 mg/m^3) 6 hours/day, 5 days/week for three months	All rats survived until the end of the study. No exposure related effect on final mean body weights and body weight gains, clinical findings or gross lesions. Some hematology or clinical chemistry data changes observed (RBC count, hemoglobin concentrations, haematocrit, total protein, albumin, globulin, urea nitrogen concentrations) but transient and returned to chamber control levels by week 14. ↗ of several hepatic enzymes activity (alkaline phosphatase, sorbitol dehydrogenase, alanine aminotransferase). Changes mostly transient. Relative kidney weights of 6.25, 12.5 and 100 ppm males and absolute and relative kidney weights of 12.5 ppm or greater females significantly greater than those of the controls. Significantly lower sperm motility (approximately 5% less than chamber controls) in male rats exposed to 100 ppm, with lower spermatid/g testis and total spermatid/testis values (15% and 16%, respectively, compared to chamber controls). At necropsy, rats did not display any histopathologic change in the contralateral organ (however, poor fixation quality of the rat testes). No VDC-related	NTP, 2015 Klimisch 1

		changes in estrous cyclicity in female rats. Microscopic lesions of the nose noted in both sexes of rats. A combination of lesions in the nasal epithelium composed of **olfactory epithelium atrophy, mineralization, necrosis and turbinate atrophy** observed with generally increasing severity with increasing exposure. No-effect level not observed, although most of the lesions minimal in rats exposed to 12.5 ppm or less: **olfactory epithelium mineralization** already significant at 6.25 ppm in both sexes and **atrophy** in males. In the liver of male rats, minimal to mild **centrilobular cytoplasmic alteration** significantly ↗ at 12.5 ppm or greater (not observed in female rats). In females, **cytoplasmic vacuolization** observed at 50 and 100 ppm. LOAEC: 25 mg/m^3 (0.025 mg/L) NOAEC: / **Category 1** (GV range for STOT RE1 (vapour): ≤ 0.2 mg/L/6h/day)	
Chronic study SD rats similar to OECD guideline 451/452 85-86 rats/sex/dose GLP: not stated	Vinylidene chloride purity 99% 0, 10, 40 ppm for 5 weeks (0, 40, 160 mg/m^3), then 0, 25, 75 ppm (0, 100, 300 mg/m^3) 6 h/d, 5 d/wk for 18 months Interim sacrifices at 1, 6 and 12 months Sacrifice at 18 and 24 months	No exposure-related changes in mortality, appearance and demeanour, body weight, clinical chemistry determinations, haematological evaluations, urinalysis, or cytogenetic evaluation of bone marrow preparations. Minimal hepatocellular fatty change in the mid-zonal region of the hepatic lobule observed in both male and female in the 100 and 300 mg/m^3 groups at the 6-month interim sacrifice (male: control: 0/5; 100 mg/m^3: 1/5; 300 mg/m^3: 4/5; female: control, 0/5; 100 mg/m^3: 2/5; 300 mg/m^3: 4/5). Fatty change also observed at the 12-month sacrifice, but no indication of progression of severity (male: control: 0/5; 100 mg/m^3: 3/5; 300 mg/m^3: 5/5; female: control: 0/5; 100 mg/m^3: 5/5; 300 mg/m^3: 5/5). At the 18-month sacrifice, incidence of this change no longer ↗ in male rats (control: 0/27; 100 mg/m^3: 0/25; 300 mg/m^3: 1/27). However, the change persisted in female rats (control: 0/16; 100 mg/m^3: 6/29; 300 mg/m^3: 7/20). In female rats, fatty change statistically significant ($P < 0.05$) only at the highest exposure. During the last 6 months of the study, after exposure had been discontinued, effect no longer discernible (male: control: 0/46; 100 mg/m^3, 1/47; 300 mg/m^3: 0/51; female: control: 0/49; 100 mg/m^3: 0/46; 300 mg/m^3: 1/48). Since the **hepatocellular mid-zonal fatty change** is minimal, reversible and did not result in altered organ weight, clinical chemistry changes diagnostic for liver damage, or any obvious decrement in liver function, it is not considered as a severe or significant toxic effect. LOAEC(female; most sensitive sex): 300 mg/m^3 (0.3 mg/L) NOAEC (female): 100 mg/m^3 **No classification** (GV range for STOT RE (vapour): ≤ 3 mg/L/6h/day after 1 month, ≤ 0.5 mg/L/6h/day after 6 months, ≤ 0.25 mg/L/6h/day after 12 month, ≤ 0.17 mg/L/6h/day after 18 months)	Rampy L.W. et al., 1977 / Quast J.F. et al, 1986 Klimisch 2
Exposure for 12 months CD rats 36 rats/sex/dose	Vinylidene chloride purity 99% 0, 55 ppm (220 mg/m^3)	No remarkable adverse signs seen in any rats during the first 7 months. One female rat exposed terminated before the end of study. Body weights of the female rats exposed to VDC generally less than that of the female controls after the 4th week and those of the males generally less than that of the male controls after the 24th	Lee C.C. et al., 1977 Klimisch 3

GLP: not stated	6 h/d, 5 days per week for 12 months Interim terminations of 4 rats after 1, 2, 3, 6, and 9 months	week. No persistent change in the following examinations of male and female rats exposed to VDC: hematology, clinical blood chemistry, pulmonary macrophage count, cytogenic analysis of bone marrow culture, x-ray examination of extremities, collagen contents in liver and lung, serum ALA synthetase, urinary ALA level and serum α-fetoprotein. A mild to markedly severe focal, **disseminated vacuolization**, probably **fatty change**, observed in livers of most of the rats treated. LOAEC: 220 mg/m^3 (0.22 mg/L) **Category 2** (GV range for STOT RE2 (vapour): 0.6-3 mg/L/6h/day after 1 month, 0.3-1.5 mg/L/6h/day after 2 months, 0.2-1.0 mg/L/6h/day after 3 months, 0.1-0.5 mg/L/6h/day after 6 months, 0.06-0.3 mg/L/6h/day after 9 months, 0.05-0.25 mg/L/6h/day after 12 months). There is no indication if the liver changes were already seen before terminal sacrifice. Thus GV for a 12-month study was considered.	
Exposure for 52 weeks GLP: not stated SD rats 30 rats/sex/dose 100 rat/sex in control group Only the early results available. Except histology examinations for tumours analysis, no information on other examinations (hematology, clinical chemistry, urine analysis, body and organ weight…) or statistical methods available.	Vinylidene chloride Purity: 99.9% 0, 10, 25, 50, 100, 200-150 ppm (40, 100, 200, 400, 600-800 mg/m^3) Exposure: 4 h/d, 4-5 days/week for 52 weeks	The highest dose level of 200 ppm was reduced to 150 ppm after 2 exposures because of high toxicity. Hepatocyte vacuolization, cloudy swelling, fatty degeneration, necrobiosis and necrosis found in some animals, treated as in control, but observed more frequently in 150-200 ppm group (57.6%) than in control (20.5%). LOAEC: 600-800 mg/m^3 (0.6-0.8 mg/L) NOAEC: 400 mg/m^3 **No classification** (GV range for STOT RE (vapour) ≤ 0.25 mg/L/6h/day after 12 month-exposure)	Maltoni C. et al., 1977 Maltoni C. et al., 1984 Klimisch 3
Subchronic study similar to OECD guideline 413 GLP: not stated Rats (Long Evans or Sprague Dawley)	Vinylidene chloride Purity ≥ 98% 0, 20 ± 2.1, 61 ± 5.7, 101 ± 4.4, 189 ± 6.2 (C) and 395 ± 32 (R) mg/m^3	Repeated exposure: No deaths, no visible signs of toxicity and no haematological or histopathological changes attributed to exposure to VDC. NOAEC = 395 mg/m^3 Continuous exposure: Varying degrees of growth depression found in all exposures, but significant only at 189 mg/m^3. No significant haematological alterations and serum urea nitrogen levels within control limits. Significant elevations of serum glutamic–pyruvic transaminase and liver alkaline phosphatase activities found (a 3-	Prendergast et al., 1967 Klimisch 3

15-45 rats/sex/dose	Exposure: Continuous (C): 90d Repeated (R): 30 exposures, 8h/day; 5d/week	fold and 1.75-fold increase, respectively) at 189 mg/m^3, but not at 20 mg/m^3 (intermediate exposures not tested). Histopathological examination of liver revealed damages at 189 mg/m^3 (**fatty metamorphosis, focal necrosis, haemosiderosis deposition, lymphocytic infiltration, bile duct proliferation and fibrosis**). Sections of kidney from all rats showed **nuclear hypertrophy of the tubular epithelium.** No detectable liver or kidney damage observed at 101 mg/m^3 or less. LOAEC = 189 mg/m^3 (0.189 mg/L) NOAEC = 101 mg/m^3 **Category 1** (GV range for STOT RE 1: ≤0.2 and ≤0.6 mg/L/6h/day after 3 month- and 1-month exposure, respectively).	
90-day subchronic neurotoxicity study similar to OECD guideline 413 and 424 GLP: yes Wistar rats 10 rats/sex/dose	Vinylidene chloride Purity: 99.97% 0, 100 ppm (400 mg/m^3) Nose only exposure: 6 h/d, 5 d/week for 90 days	All animals survived until scheduled necropsy. Slight but significant reduction ($p \leq 0.01$) in body weight in female rats exposed to VDC. This reduction ↗ with time during exposure, and considered related to the treatment. However, no ↘ in body weight gain. No significant difference in food consumption between groups. Regarding the neurobehaviour, most functional domains of the nervous system tested appeared unaffected. In the neuromuscular domain, small differences observed in the parameters gripstrenght and landing footsplay between groups. In male, after 13 weeks of exposure, significantly lower gripstrength and footsplay observed in the exposed group. Both parameters appear to show a gradual significant ↘ with time during exposure period. In females, gradual ↘ observed over time during exposure period for VDC group did not reach the level of significance. Small adverse effect on the neuromuscular domain, however, cannot be ruled out. Numerical data were not available to the DS. No significant change in liver weights (absolute or relative). No macroscopic changes observed. Microscopic examination of the liver: very slight to slight mononuclear cell aggregates and **necrotic hepatocytes, hepatocellular vacuolation and hepatocellular hypertrophy** in both sexes in exposed group as well as in control group. Statistically significant hepatocellular hypertrophy observed mainly in the male rats of the VDC group and occurred mainly centrilobularly. Regarding neuropathology, microscopic examination indicated significant incidence of axonal degeneration in the sural nerve in female rats and in the tibial nerve of male rats (within the range of the normal background pathology of rats in this strain and age [data not available]). LOAEC: 400 mg/m^3 (0.4 mg/L) **Category 2** (GV range for STOT RE2 (vapour): 0.2-1.0 mg/L/6h/day after 90 day-exposure).	Anonymous, 2004 Klimisch 4
Subacute study similar to OECD guideline 412 GLP: no SD rats	Vinylidene chloride Purity: 99.9% 0, 30 and 100 ppm (120 and 400 mg/m^3)	No effect on mortality, no clinical signs observed. No data available on food consumption. No adverse effect observed either in haematology or clinical chemistry analysis. No significant variation observed in body weight or body weight gain. Variations of absolute or relative organ weights observed: ↗ of absolute kidney weight at 100 ppm and ↘ of adrenal gland at 30	Anonymous, 1979h Klimisch 4

20 rats/sex/dose	whole body exposure: 6 hours/day, 5 days/week for 6 weeks (30 days of treatment).	ppm only in females. ↗ of relative kidney weight found in males and females at 100 ppm, and only in females at 30 ppm. ↗ of the relative liver weight observed in females at 100 ppm, and ↘ of the adrenal gland in females at 30 ppm. None of these findings were statistically significant. No relevant histopathology findings observed. Several pulmonary changes, the spleen and the kidneys show minor variations in the normal morphological field. Others isolated cases show insignificant changes (heart, trachea, gland submandibulaire, thyroid). These results were considered related to the killing or insignificant. NOAEC: 400 mg/m^3 = (0.4 mg/L) **No classification possible** (GV range for STOT RE2 (vapour): 0.6-3.0 mg/L/6h/day and for STOT RE 1 ≤ 0.6 mg/L/6h/day after 30 day-exposure).	
Subacute study similar to OECD guideline 412 GLP not stated Alderley Park specific-pathogen-free rats 4 rats/sex/dose	Vinylidene chloride Purity not stated 0, 200, 500 ppm (0, 800, 2000 mg/m^3) Whole body exposure 6h/d, 5 d/week, for 4 weeks.	500 ppm: nose irritation, retarded weight gain and **liver cell degeneration observed.** 200 ppm: only slight nose irritation and no significant findings noted at the autopsy. No other information available. LOAEC = 2000 mg/m^3 (2 mg/L) NOAEC = 800 mg/m^3 **Category 2** (GV range for STOT RE2 (vapour): 0.6-3.0 mg/L/6h/day after 30 day-exposure).	Gage et al, 1970 Klimisch 4
Studies on mice			
Subacute study GLP compliant B6C3F1/N mice 5 mice/sex/dose Whole body inhalation	Vinylidene chloride Purity > 99.9% 0, 25, 50, 100, 200, 400 ppm (0, 100, 200, 400, 800, 1600 mg/m^3) 6 hours/day, 5 days/week for 2 weeks	All male mice exposed to 100 ppm or greater died within the first 4 days of exposure. All females exposed to 200 or 400 ppm were found dead following exposure on day 1. One 50 ppm male and one 100 ppm female removed dead before exposure on day 5. Mean body weight gains of 25 and 50 ppm males significantly less than that of the chamber controls; final mean body weights of these groups 8% and 7% less, respectively, than that of the chamber control group. Two of five 50 ppm males and all 100 ppm males were lethargic. Abnormal breathing in one of five 50 ppm males and four of five 100 ppm males. All 100 ppm female mice became thin, while one female exposed at this level also became lethargic, developed tremors and was breathing abnormally. In all surviving groups of exposed females, absolute and relative lung weights significantly greater than those of the chamber controls. Absolute and relative liver weights of 50 and 100 ppm females and relative liver weights of 25 ppm females and 25 and 50 ppm males significantly greater than those of the chamber controls. Gross lesions observed at 100 ppm: pale or mottled livers in one male and one female, and pale kidney in one male mouse that survived more than 1 day of exposure. Nose: **minimal necrosis of the respiratory epithelium** in all early-death male and female mice. Liver: necrosis in all males and females exposed to 100 ppm or greater, and in one male exposed to 50 ppm; in addition, regeneration in the four 100 ppm females surviving to the end of	NTP, 2015 Klimisch 1

		study. **Hepatic necrosis** moderate to marked in all early-death mice exposed to 100 ppm or greater and minimal in the one 50 ppm male. Minimal in the four 100 ppm female mice surviving to terminal kill. Kidney: renal tubule necrosis and granular casts in every exposed male. Incidences of marked renal tubule necrosis coincided with early deaths in all male mice exposed to 100 ppm or greater. Incidences of **minimal to moderate renal tubule necrosis and granular casts** occurred in the 25 and 50 ppm male groups. **Mild to moderate renal tubule regeneration** in 25 and 50 ppm males surviving until terminal sacrifice. LOAEC (males, most sensitive sex): 100 mg/m^3 (0.1 mg/L) NOAEC (males, most sensitive sex): / **Category 1** (GV range for STOT RE 1: ≤ 1.2 mg/L/6h/day after 2 week-exposure).	
Subchronic study similar to OECD guideline 413 GLP compliant B6C3F1/N mice 10 mice/sex/dose Whole body inhalation	Vinylidene chloride Purity > 99.9% 0, 6.25, 12.5, 25, 50, 100 ppm (0, 25, 50, 100, 200, 400 mg/m^3) 6 hours/day, 5 days/week for 3 months	Two 50 ppm males and four 100 ppm females died during the first week of the study; all other mice survived until terminal kill. Final mean body weights and body weight gains of all exposed groups of females and of males exposed to 12.5 ppm or greater significantly less than the those of the chamber control groups. No exposure-related clinical findings. Gross lesions potentially related to exposure in the lung (5/10) and liver (1/10) of 100 ppm female mice and the liver (1/10) and kidney (2/10) of 50 ppm male mice. Lung lesions included pale to white, 1 to 7 mm diameter foci; affected livers were mottled and/or red and affected kidneys were diffusely pale and/or granular. Hematology data: exposure concentration-related ↘ (≤8%) in erythrocyte counts, hemoglobin concentrations, and hematocrit values at the end of the study in 12.5, 25, and 50 ppm male mice. Female mice had ↘ erythrocyte counts (≤4% less than in males) in the 50 and 100 ppm groups, hemoglobin concentration and hematocrit value ↘ only at 50 ppm. Absolute kidney weights of all exposed groups of males significantly less than that of the chamber control group. ↗ relative liver weights at all concentrations and absolute weight at 12.5 ppm or greater in females. Absolute and relative kidney and lung weights of 100 ppm females significantly greater than those of the chamber controls. Relative to the chamber controls, male mice exposed to 25 or 50 ppm exhibited non-significant ↘ in cauda epididymis weights (18% and 10%, respectively). Males exposed to 12.5, 25, or 50 ppm had significant ↘ in total sperm/cauda epididymis. No histopathologic changes in the contralateral organ observed at necropsy. No changes in estrous cyclicity in females attributed to VDC. Kidney lesions (limited to males): renal tubule necrosis and protein cast formation in mice that experienced early death and nephropathy in those surviving to terminal kill. Marked necrosis of the renal tubules and protein cast formation occurred in two 50 ppm males. **Minimal to moderate nephropathy** occurred in the 12.5, 25, and 50 ppm male groups. Laryngeal lesions: necrosis and respiratory epithelium hyperplasia and squamous metaplasia. Necrosis was minimal and only seen in early death 100 ppm females. Respiratory epithelium hyperplasia	NTP, 2015 Klimisch 1

		occurred in most 100 ppm females and **respiratory epithelium squamous metaplasia** occurred in a few males and many females exposed to 25 ppm or greater, with slight ↗ in severities and incidences in the female mice. Nonneoplastic lesions of the liver included necrosis in male and female mice and centrilobular hepatocyte hypertrophy in female mice. Necrosis was marked in early death 100 ppm females and mild in early death 50 ppm males: from piecemeal necrosis (individual hypereosinophilic hepatocytes with nuclear pyknosis and karyolysis) to more extensive necrosis, characterized by a hypereosinophilic coagulum within the centrilobular to midzonal regions that often extended into periportal areas. Hepatic necrosis was not evident in the 50 ppm mice that survived to terminal kill. Mild to moderate centrilobular hepatocyte hypertrophy observed in six 100 ppm female mice. Exposure-related lung lesions limited to 100 ppm female mice: bronchial epithelium necrosis in 4 early-death females and 2 females surviving to terminal kill and histiocytic inflammation in all of the females surviving to terminal kill. Minimal to moderate necrosis of the nasal respiratory epithelium (all early-death female) and minimal turbinate atrophy (4 females) in females exposed to 100 ppm. Male mice did not develop exposure-related nasal lesions. LOAEC (males): 50 mg/m^3 (0.05 mg/L) NOAEC (males): 25 mg/m^3 **Category 1** (GV range for STOT RE 1: ≤ 0.2 mg/L/6h/day after 90 day-exposure).	
Chronic study similar to OECD guideline 452 GLP: not stated Albino CD-1 mice 36 mice/sex/dose	Vinylidene chloride Purity: 99% 0, 55 ppm (220 mg/m^3) Exposure: 6 h/d, 5 days/week, 12 months Interim sacrifices at 1, 2, 3, 6 and 9 months	Two males exposed to VDC died on the 13th day and replaced with healthy mice. Thereafter, all mice appeared in good health. Two males terminated during the ninth month and one female during the 10th month. They all had tumors in the liver. Weight gains of animals exposed to VDC comparable to controls. No persistent change found in the following laboratory results of the male and female mice exposed to VDC as compared with those of the respective controls: hematology, clinical blood chemistry, cytogenic analysis of bone marrow cultures, x-ray examinations of extremities, and serum α-fetoprotein. Lesions in Early Deaths (two males exposed to VDC for 13 days): acute toxic hepatitis, characterized by focal to marked congestion, and marked diffused coagulation type necrosis of hepatocytes beginning in the centrilobular area. Marked tubular necrosis characterized by pyknosis and eosinophilic granulation of the cytoplasma in the renal cortex was also observed. Lesions in Late Deaths (scheduled or unscheduled): several changes in the liver. Enlarged and basophilic hepatocytes with enlarged nuclei, many of which had large round eosinophilic inclusions; mitotic figures or polyploidy; microfoci of mononuclear cells; **focal degeneration and necrosis**. Incidence and severity of these lesions progressed with lengths of exposure. Some of these mice also had hemangiosarcoma. LOAEC: 220 mg/m^3 (0.220 mg/L) **Category 2** (GV range for STOT RE2 (vapour): 0.6-3 mg/L/6h/day after 1 month, 0.3-1.5 mg/L/6h/day after 2 months,	Lee C.C. et al., 1977 Klimisch 3

		0.2-1.0 mg/L/6h/day after 3 months, 0.1-0.5 mg/L/6h/day after 6 months, 0.06-0.3 mg/L/6h/day after 9 months, 0.05-0.25 mg/L/6h/day after 12 months).	
Chronic study similar to OECD guideline 452 GLP: not stated Swiss mice First part: control group: 100 mice/sex 30 mice/sex/dose Second part: control group: 90 mice/sex 25 ppm: 120 mice/sex	Vinylidene chloride Purity > 99.9% 0, 10, 25, 50, 100, 200 ppm (40, 100, 200, 400, 800 mg/m^3) Exposure: 4 h/d, 4-5 days/week for 52 weeks Observation up to 121 weeks	Exposure to 50, 100 and 200 ppm had to be withdrawn due to high mortality and severe toxic effects. Regressive changes (hepatocyte vacuolization, cloudy swelling, fatty degeneration, necrobiosis and necrosis) and amyloidosis in the liver and regressive changes (cloudy swelling and necrosis), amyloidosis of glomeruli and chronic nephritis of kidneys in both control and exposed animals: no correlation emerges between these changes and exposure. NOAEC: / **No classification possible (effects reported in both control and exposed animals)**	Maltoni C. et al., 1977 Maltoni C. et al., 1984 Klimisch 3
Studies on others animals			
Chronic study similar to OECD guideline 452 GLP: not stated Chinese hamsters 30/sex/dose 17-18/sex in control group	Vinylidene chloride Purity: 99.9% 0, 10, 25 ppm (40, 100 mg/m^3) Exposure: 4 h/d, 4-5 days/week for 52 weeks	No significant changes observed LOAEC = / NOAEC = 100 mg/m^3 **No classification possible**	Maltoni C. et al., 1977 Maltoni C. et al., 1984 Klimisch 3
Subchronic study similar to OECD guideline 452 GLP: not stated Hartley Guinea Pigs 15-45 guinea pigs/sex/dose, except control group	Vinylidene chloride Purity ≥ 98% 0, 20 ± 2.1 (mean of 3 run), 61 ± 5.7, 101 ± 4.4, 189 ± 6.2 (C) and 395 ± 32 (R) mg/m^3 Exposure: Continuous (C): 90d Repeated (R): 30 exposures, 8h/day; 5days/week	Repeated exposure: No deaths, no visible signs of toxicity and no haematological or histopathological changes attributed to exposure to VDC. NOAEC = 395 mg/m^3 Continuous exposure: Mortality was 2/314, 2/45, 3/15, 3/15, and 7/15 in guinea-pigs in the 0, 20, 61, 101, or 189 mg/m^3 exposure groups, respectively. No visible signs of toxicity in any surviving animals. Varying degrees of growth depression found in all exposures, but significant only at 189 mg/m^3. No significant haematological alterations, and serum urea nitrogen levels within control limits in all exposures. Significant elevations of serum glutamic–pyruvic transaminase and liver alkaline phosphatase activities (a 7-fold and 2.4-fold increase, respectively) at 189 mg/m^3, but not at 20 mg/m^3 (intermediate exposures not tested). No detectable liver or kidney damage observed. NOAEC = 189 mg/m^3 (0.189 mg/L) **No classification possible**	Prendergast et al., 1967 Klimisch 3

Subchronic study similar to OECD guideline 413 GLP: not stated Squirrel monkeys 3 to 21/sex/dose, except in control group	Vinylidene chloride Purity ≥ 98% 0, 20 ± 2.1, 61 ± 5.7, 101 ± 4.4, 189 ± 6.2 (C) and 395 ± 32 (R) mg/m^3 Exposure: Continuous (C): 90d Repeated (R): 30 exposures, 8h/day; 5days/week	Repeated exposure: No deaths, no visible signs of toxicity, and no haematological or histopathological changes attributed to exposure to VDC. NOAEC = 395 mg/m^3 Continuous exposure: Mortality was 1/57, 1/21, 0/9, 2/3 and 3/9 in the 0, 20, 61, 101 or 189 mg/m^3 exposure groups, respectively. Varying degrees of growth depression found in all exposures, but significant only at 189 mg/m^3. Test animals exhibited no significant haematological alterations, and serum urea nitrogen levels were within control limits in all exposures in which determinations were made. Histopathological examination of liver revealed damage at 189 mg/m^3. Effects observed included **fatty metamorphosis, focal necrosis, haemosiderosis deposition, lymphocytic infiltration, bile duct proliferation and fibrosis**. No detectable liver or kidney damage observed at 101 mg/m^3 or less. LOAEC = 189 mg/m^3 (0.189 mg/L) NOAEC = 101 mg/m^3 **Category 1** (GV range for STOT RE 1: ≤ 0.2 and ≤ 0.6 mg/L/6h/day after 3 month- and 1-month exposure, respectively).	Prendergast et al., 1967 Klimisch 3
Subchronic study similar to OECD guideline 413 GLP: not stated New Zealand albino rabbits 3/sex/dose	Vinylidene chloride Purity ≥ 98% 0, 101 ± 4.4, (C) and 395 ± 32 (R) mg/m^3 Exposure: Continuous (C): 90d Repeated (R): 30 exposures, 8h/day; 5days/week	Repeated exposure: No deaths, no visible signs of toxicity, and no haematological or histopathological changes attributed to exposure to VDC. NOAEC = 395 mg/m^3 Continuous exposure: No visible signs of toxicity in any surviving animals. Varying degrees of growth depression found in all exposures, but significant only at 189 mg/m^3. No significant haematological alterations, and serum urea nitrogen levels within control limits in all exposures in which determinations were made. No detectable liver or kidney damage observed. LOAEC = / NOAEC = 189 mg/m^3 (0.189 mg/L) **No classification possible**	Prendergast et al., 1967 Klimisch 3
Subchronic study similar to OECD guideline 413 GLP: not stated Beagle dogs 2 dogs/sex/dose	Vinylidene chloride Purity ≥ 98% 0, 20 ± 2.1, 61 ± 5.7, 101 ± 4.4, 189 ± 6.2 (C) and 395 ± 32 (R) mg/m^3 Exposure: Continuous (C): 90d Repeated (R): 30 exposures, 8h/day; 5days/week	Repeated exposure: No deaths, no visible signs of toxicity, and no haematological or histopathological changes attributed to exposure to VDC. NOAEC = 395 mg/m^3 Continuous exposure: No visible signs of toxicity in any surviving animals. Varying degrees of growth depression found in all exposures, but significant only at 189 mg/m^3. Test animals exhibited no significant haematological alterations, and serum urea nitrogen levels were within control limits in all exposures in which determinations were made. Histopathological examination of liver revealed damage at 189 mg/m^3 (**fatty metamorphosis, focal necrosis, haemosiderosis deposition, lymphocytic infiltration, bile duct proliferation, and fibrosis**). No detectable liver or kidney damage observed at 101 mg/m^3 or less. LOAEC = 189 mg/m^3 (0.189 mg/L) NOAEC = 101 mg/m^3 **Category 1** (GV range for STOT RE 1: ≤ 0.2 and ≤ 0.6 mg/L/6h/day after 3 month- and 1-month exposure, respectively).	Prendergast et al., 1967 Klimisch 3

Effects in bold are those considered for classification purpose

10.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Table 24: Summary of effective dose for toxicity studies available

Species	Study reference	Effective dose (mg/kg bw/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Oral route					
Rats	Quast et al., 1983	9 mg/kg bw/d	2 years	72 mg/kg bw/d	**No classification**
	NTP, 1982	5 mg/kg bw/d	104 weeks	40 mg/kg bw/d	**Category 2**
	NTP, 1982	100 mg/kg bw/d	13 weeks	/	**Category 2**
Mice	NTP, 1982	10 mg/kg bw/d	104 weeks	80 mg/kg bw/d	**Category 2**
	NTP, 1982	100 mg/kg bw/d	13 weeks	/	**Category 2**
Dogs	Quast et al., 1983	/	97 days	/	**No classification possible**
Inhalation route					
Rats	NTP, 2015	100 mg/m^3 (0.1 mg/L)	2 weeks	0.015 mg/L	**Category 1**
	NTP, 2015	25 mg/m^3 (0.025 mg/L)	13 weeks	/	**Category 1**
	Rampy L.W. et al., 1977 / Quast J.F. et al, 1986	300 mg/m^3 (0.3 mg/L)	18 months	1.8 mg/L	**No classification**
	Lee C.C. et al., 1977	220 mg/m^3 (0.22 mg/L)	12 months	0.88 mg/L	**Category 2**
	Maltoni C. et al., 1977 Maltoni C. et al., 1984	600-800 mg/m^3 (0.6-0.8 mg/L)	52 weeks	2.4-3.2 mg/L	**No classification**
	Prendergast et al., 1967	189 mg/m^3 (0.189 mg/L)	13 weeks	/	**Category 1**
	Anonymous, 2004	400 mg/m^3 (0.4 mg/L)	13 weeks	/	**Category 2**
	Anonymous, 1979f	/	6 weeks	/	**No classification possible**
	Gage et al, 1970	2000 mg/m^3 (2 mg/L)	4 weeks	0.62 mg/L	**Category 2**
Mice	NTP, 2015	100 mg/m^3 (0.1 mg/L)	2 weeks	0.15 mg/L	**Category 1**

Species	Study reference	Effective dose (mg/kg bw/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
	NTP, 2015	50 mg/m^3 (0.05 mg/L)	13 weeks	/	**Category 1**
	Lee C.C. et al., 1977	220 mg/m^3 (0.22 mg/L)	12 months	0.88 mg/L	**Category 2**
	Maltoni C. et al., 1977 Maltoni C. et al., 1984	/	52 weeks	/	**No classification possible**
Hamster	Maltoni C. et al., 1977 Maltoni C. et al., 1984	/	52 weeks	/	**No classification possible**
Guinea Pigs	Prendergast et al., 1967	189 mg/m^3 (0.189 mg/L)	13 weeks	/	**No classification possible**
Monkeys	Prendergast et al., 1967	189 mg/m^3 (0.189 mg/L)	13 weeks	/	**Category 1**
Rabbits	Prendergast et al., 1967	/	13 weeks	/	**No classification possible**
Dogs	Prendergast et al., 1967	189 mg/m^3 (0.189 mg/L)	13 weeks	/	**Category 1**

Oral route

Studies in rodents available by oral route (NTP studies on rats and mice (1982), Quast et al., 1983)) are of equivalent good quality. In all studies, adverse effects were observed on the liver (such as necrosis, hepatocytomegaly, portal and subcapsular fibrosis, bile duct hyperplasia, hepatocellular atrophy, congestion, fatty metamorphosis). Some effects in the kidney (chronic inflammation) were also reported in the chronic NTP study (1982) in rats. Studies in rats and mice lead to a classification in category 2 (CLP Regulation guidance value: $10 < C \leq 100$ mg/kg bw for a 90-day study). In contrast, no severe adverse effect was reported in dogs in a 97-day study at doses up to 25 mg/kg bw/day.

Inhalation route

By inhalation route, many more studies are available in various species, but only the recent NTP studies (NTP, 2015) are of high quality. In the two-week studies, 5 animals/sex were exposed to 0, 25, 50, 100, 200, 400 ppm (0, 100, 200, 400, 800, 1600 mg/m^3). In the three-month studies, 10 animals/sex were exposed to 0, 6.25, 12.5, 25, 50, 100 ppm (0, 25, 50, 100, 200, 400 mg/m^3). The two-year studies described in the carcinogenicity section (10.7) are difficult to use for the STOT-RE classification, as some classical parameters of repeated toxicology studies are not investigated (as haematological or biological chemistry), but, more importantly, even the lowest concentrations used in these studies are higher than the threshold

values for classification as STOT RE 1. Therefore, the high quality 90-day studies were used in priority as they can be directly compared to guidance values for STOT RE classification (i.e. without extrapolation of duration). Nevertheless, the two-year studies are described thoroughly in the carcinogenicity section, and it can be noted that the target organs of VDC in these studies are consistent with studies of lower duration.

In rats, adverse effects were described on the liver, characterised by centrilobular cytoplasmic alteration from mild to moderate severity in the two-week study from 25 ppm (0.1 mg/L), and from minimal to mild severity in the three-month study from 12.5 ppm (0.05 mg/L) (table 25). Effects in the nose were also reported in the 3-month study, at all tested concentrations (from 6.25 ppm (0.025 mg/L)) including olfactory epithelium atrophy, mineralization, necrosis and turbinate atrophy, although minimal in severity. Severity increased with increased concentrations. Effects on kidneys were also observed (renal tubules casts) but at higher doses.

Table 25 Incidence of lesions leading to category 1 classification in rats in the 2-week and 3-month NTP inhalation study (NTP, 2015)

2-week study		Control	25 ppm 0.1 mg/L	50 ppm 0.2 mg/L	100 ppm 0.4 mg/L	200 ppm 0.8 mg/L	400 ppm 1.6 mg/L
Centrilobular cytoplasmic alteration	Males	0	4* (2.8)	5** (3.0)	5** (3.0)	0[a]	0
	Females	0	5** (2.4)	5** (3.0)	5** (2.6)	0	0
Renal Tubule, Casts	Males	0	–	–	0	5** (3.2)	4* (2.5)
	Females	0	–	–	0	5** (3.0)	5** (3.2)
3-month study		Control	6.25 ppm 0.025 mg/L	12.5 ppm 0.05 mg/L	25 ppm 0.1 mg/L	50 ppm 0.2 mg/L	100 ppm 0.4 mg/L
Centrilobular cytoplasmic alteration	Males	1 (1.0)	1 (1.0)	6* (1.7)	10** (1.8)	10** (2.0)	10** (1.9)
Cytoplasmic vacuolization	Females	0	0	0	0	10** (1.1)	10** (1.0)
Olfactory Epithelium, Atrophy	Males	0	4* (1.0)	10** (1.0)	10** (1.7)	10** (2.2)	10** (2.7)
	Females	0	2 (1.0)	10** (1.0)	10** (1.3)	10** (1.7)	10** (2.4)
Olfactory Epithelium, Mineralization	Males	0	10** (1.3)	10** (2.0)	10** (2.9)	10** (3.0)	10** (2.6)
	Females	0	5* (1.0)	9** (1.3)	10** (1.9)	10** (2.1)	10** (2.3)
Olfactory Epithelium, Necrosis	Males	0	2 (1.0)	6** (1.0)	9** (1.0)	7** (1.7)	10** (1.6)
	Females	0	1 (1.0)	3 (1.3)	6** (1.5)	10** (2.2)	10** (1.6)
Turbinate, Atrophy	Males	0	0	10** (1.0)	10** (2.0)	10** (2.2)	10** (3.0)
	Females	0	0	10** (1.0)	10** (2.0)	10** (2.2)	10** (3.0)

*Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact test.
**$P \leq 0.01$.
Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[a] Rats at 200 and 400 ppm did not have cytoplasmic alteration, but rather centrilobular necrosis, consistent with a more severe stage of hepatocellular damage.

The LOAEC of these two rats studies are 0.1 and 0.025 mg/L for subacute and subchronic durations, respectively.

In mice, effects were consistently observed in kidney, liver and nose. Critical effects were reported in the kidneys in males, with renal tubule necrosis, granular casts and renal tubule regeneration in the 2-week study from 25 ppm (0.1 mg/L), and nephropathy (described by NTP as being "*composed of minimal to mild tubule necrosis and cast formation; renal tubule regeneration; mild inflammatory infiltrates of lymphocytes, macrophages, and neutrophils within the interstitium and subcapsular areas; and occasional tubule mineralization*") in the 3-month study from 12.5 ppm (0.05 mg/L) (Table 26). In the two studies, relevant effects were also described in the nose, with necrosis of the respiratory epithelium or respiratory epithelium squamous metaplasia and in the liver (same findings as reported in rats), but at higher doses.

Table 26: Incidence of lesions leading to category 1 classification in mice in the 2-week and 3-month NTP studies (NTP, 2015)

2-week study		Control	25 ppm 0.1 mg/L	50 ppm 0.2 mg/L	100 ppm 0.4 mg/L	200 ppm 0.8 mg/L	400 ppm 1.6 mg/L
Renal Tubule, Necrosis	Males	0	5** (1.2)	5** (1.6)	5** (4.0)	5** (4.0)	5** (4.0)
Cast Granular		0	5** (1.8)	5** (2.2)	5** (3.0)	5** (4.0)	5** (4.0)
Renal Tubule, Regeneration		0	5** (2.8)	4* (3.0)	0	0	0
Respiratory Epithelium, Necrosis		0	0	1 (1.0)	5** (1.0)	5** (1.0)	5** (1.0)
Liver necrosis	Males	0	0	1 (1.0)	5** (3.0)	5** (4.0)	5** (4.0)
	Females	0	–	0	5** (1.6)	5** (4.0)	5** (4.0)
3-month study		Control	6.25 ppm 0.025 mg/L	12.5 ppm 0.05 mg/L	25 ppm 0.1 mg/L	50 ppm 0.2 mg/L	100 ppm 0.4 mg/L
Nephropathy	Males	0	0	5* (1.2)	10** (1.9)	8** (2.5)	/
Respiratory Epithelium, Metaplasia, Squamous	Males	0	0	0	1 (1.0)	4* (1.0)	–
	Females	1 (1.0)	0	1 (2.0)	3 (1.3)	9** (1.8)	7** (2.4)

*Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact test.
**$P \leq 0.01$.
Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

The LOAEC of these two mice studies are therefore based on renal effects, and are 0.1 and 0.05 mg/L for subacute and subchronic durations, respectively.

Other available studies described in table 23 are of lower quality (use of only one dose, lack of details…). However, qualitatively they support the results of the NTP studies, in particular by identifying the liver and kidney as main target organs.

10.10.2 Comparison with the CLP criteria

Guidance values (GV) are provided to assist classification of a substance in a category according to the level at which occur significant effect (based on a 90-day study):
For oral route:

- Category 1 : GV ≤ 10 mg/kg bw/day;
- Category 2 : 10 mg/kg bw/day < GV ≤ 100 mg/kg bw/day

For inhalation:

- Category 1 : GV ≤ 0.2 mg/L;
- Category 2 : 0.2 mg/L < GV ≤ 1.0 mg/L

According to the CLP criteria, "*Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations*". No human data is available. Animal studies are therefore to be used. Reliable data on experimental animals after repeated exposure to VDC is available for oral and inhalation routes.

According to the ECHA CLP guidance (2017), "*The final classification based on non human data will be the most severe classification of the three routes.*" Regarding the data described above, inhalation appears to be the most damaging route of exposure for VDC, the available oral studies leading to a classification in category 2 at best, whereas several studies by inhalation, and particularly the most reliable ones (NTP, 2015) lead to a classification in category 1. Classification of VDC should therefore be based on studies performed via inhalation route.

The four studies in rats and mice performed by the NTP in 2015 (two-week and 13-week) are studies of high quality, that can be used as key studies to base the classification of VDC.

In the 2-week rats study, the critical adverse effects were described as centrilobular cytoplasmic alteration from minimal to mild severity which occur from 0.1 mg/L. NTP considered this alteration "*likely represents a form of hepatocellular degeneration, because rats exposed to 200 and 400 ppm did not have cytoplasmic alteration, but rather centrilobular necrosis consistent with a more severe stage of hepatocellular damage*".
In the 13-week rats study, the critical adverse effect was an atrophy of the olfactory epithelium occurring from 0.025 mg/L. NTP described this alteration as "*decrease in the number of olfactory epithelial cells lining the turbinates, usually in the dorsal meatus of Level III, and by replacement with a single layer of*

respiratory-type epithelium (metaplasia). This lesion was often associated with a corresponding decrease in nerve fibers and glands in the underlying lamina propria".

In mice, the critical effect observed was a nephropathy which occur from 0.05 mg/L, described by NTP as being "*composed of minimal to mild tubule necrosis and cast formation; renal tubule regeneration; mild inflammatory infiltrates of lymphocytes, macrophages, and neutrophils within the interstitium and subcapsular areas; and occasional tubule mineralization*".

According to the ECHA CLP guidance (2017), "*STOT-RE is assigned on the basis of findings of 'significant' or 'severe' toxicity. In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature which significantly impact on health*". According to this definition, liver alterations can therefore be considered as significant effects as they indicate functional disturbance of the organ. In the same way, nephropathy in mice can also be considered as a significant effect. The nose effects described in these studies (olfactory epithelium necrosis, olfactory epithelium and turbinate atrophy and respiratory epithelium metaplasia) occurred at all tested concentrations in the 13-week study in rats (and from 0.1 mg/L if considering effects of mild to moderate severity) and from 0.2 mg/L in the 13-week study in mice, also fulfil these definitions and thus lead to a category 1 classification.

The LOAEC associated to these critical effects are **0.025 mg/L** in the 13-week rats study and **0.05 mg/L** in the 13-week mice study. Refering to the guidance values presented above, these 13-week studies therefore lead to a classification of VDC in category 1. To be noted also that the 2-week studies (NTP, 2015) are consistent as they lead to the same category of classification.

10.10.3 Conclusion on classification and labelling for STOT RE

Regarding the data available, a classification as **STOT RE 1 H372: Causes damage to organs through prolonged or repeated exposure (liver, kidney, respiratory tract)** is warranted.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Studies both via the oral and inhalation route were assessed by the DS for STOT RE.

The DS evaluated the 13-week and 104-week NTP (1982) oral (gavage) studies in rats and mice and the Quast *et al*. (1983) studies in rats (2-year administration in drinking water) and dogs (97-day administration in gelatine capsules). In all studies, liver was recognised as

the target organ with various adverse effects of diverse severity (from changes in transaminases in dogs and cellular atypia in mice to centrilobular necrosis in rats). Chronic inflammation of the kidney was also reported in the 13-week NTP (1982) gavage study in rats. No exposure-related histopathological lesions were observed in dogs.

Studies in rats and mice showed relevant adverse effects within the CLP Regulation guidance value range for Category 2: 10 < C ≤ 100 mg/kg bw/day for a 90-day oral study.

By inhalation route, many more studies were available in various species, but only the recent NTP studies (NTP, 2015) were considered by the DS as of high quality (two-week and 13-week studies in rats and mice) and used as key studies to justify the classification proposal for VDC. In rats, adverse effects were described in the liver, characterised by centrilobular cytoplasmic alteration from minimal to moderate severity both in the two-week and in the 13-week study. Also effects in the olfactory epithelium were reported in the 13-week study at all concentrations (from 0.025 mg/L) tested. The severity of these effects was minimal at low doses and increased with increased concentrations. Effects in rat kidneys were also observed (renal tubules casts) at higher doses. In mice, effects were consistently observed in kidney, liver and nose (necrosis of the respiratory epithelium or respiratory epithelium squamous metaplasia).

The LOAEC associated to these critical effects were 0.025 mg/L in the 13-week rat study and 0.05 mg/L in the 13-week mouse study. After comparing the doses causing these effects with the CLP Regulation guidance values via the inhalation route, these 13-week studies were considered to fulfil the classification criteria for Category 1.

According to the CLP guidance (version 5, 2017), the final classification category for STOT RE based on non-human data should be the most severe classification category warranted by any of the three routes of exposure. Thus, the DS proposed a classification as STOT RE 1; H372: Causes damage to organs through prolonged or repeated exposure (liver, kidney, respiratory tract).

Comments received during consultation

During the consultation, an MSCA supported the proposed classification and the reasoning by the DS.

An Industry/Trade association disputed the proposed classification for STOT RE, as the lesions and histopathological findings used by the DS as the justification for STOT RE were considered as part of the carcinogenic activity of VDC.

The DS replied that tumours were to be assessed under carcinogenicity and had not been taken into consideration for the STOT RE classification. On the contrary, the effects such as hepatic centrilobular alteration, olfactory epithelium necrosis or even nephropathy were to be considered for classification purposes under STOT RE. Even if these effects were observed in the same organs recognised as target organs for carcinogenicity, they would not necessarily lead to the development of tumours.

Assessment and comparison with the classification criteria

Studies described in in Tables 22 and 23 of the CLH report are discussed hereafter.

Studies in rodents available by oral route (Table 22 of the CLH report) are of relatively good

quality, all rated as Klimisch 2 studies. In all studies, liver was recognised as the target organ with various adverse effects observed, namely necrosis, hepatocytomegaly, portal and subcapsular fibrosis, bile duct hyperplasia, hepatocellular atrophy, congestion and fatty metamorphosis. Some effects in the kidney, i.e., chronic inflammation, were also reported in the chronic NTP study (1982) in rats. NOAEL in rats ranged from 1 – 40 mg/kg bw/day and in mice from 2 – 40 mg/kg bw/day. The effective dose ranged between 5 and 100 mg/kg bw/day in rats and between 10 and 100 mg/kg bw/day in mice, being within the CLP Regulation guidance value range for classification in Category 2, after extrapolation to a 90-day exposure when required. In contrast, no severe adverse effects were reported in dogs in a 97-day study (Quast *et al*., 1983) at doses up to 25 mg/kg bw/day. Findings from the Quast *et al.* (1983) studies both in rats and dogs did not show effects warranting classification for STOT RE.

Regarding the inhalation route of exposure (Table 23 of the CLH report), results from the Klimisch 1 NTP (2015) whole-body inhalation studies in rats and mice are thoroughly discussed below and are considered as key evidence for classification by RAC, especially the long-term effects observed in the 13-week NTP (2015) study.

Tested concentrations in the two-week studies in rats and mice were 0, 100, 200, 400, 800, 1600 mg/m^3 and 0, 25, 50, 100, 200, 400 mg/m^3 in the 13-week (91-day) studies. Mortality and body weight gain in these studies are summarised in the following table. The findings from the high quality 90-day studies can be directly (i.e., without extrapolation of duration) compared to guidance values for STOT RE classification. In rats, no treatment-related effect in mortality and body weight gain was observed at doses where STOT RE effects were seen, indicating no general systemic toxicity. In mice, in the 13-week study, at 50 mg/m^3, no mortality was noticed in male or female mice, but reduction in body weight gain exceeded 15% for males and 20% for females compared to controls. During the study duration, though, dosed animals continued to gain weight. Therefore, any toxicity identified in target organs hereafter, could be regarded as the primary effect due to repeated exposure to VDC.

***Table**: Mortality and body weight gain at all doses in the 2-week and in the 13-week NTP (2015) study in rats and mice*

Dose (mg/m^3)	**Rats**				**Mice**			
	Males	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
	Survival		**Body weight gain (%)**		**Survival**		**Body weight gain (%)**	
2-week NTP 2015 study								
0	5/5	5/5	69.9	47.6	5/5	5/5	15.2	12.7
100	4/5	5/5	64.8	48.8	5/5	5/5	3.4	9.5
200	5/5	5/5	72.8	45.2	4/5	5/5	5.5	10.3
400	5/5	5/5	65.6	41.0	**0/5**	4/5		13.3
800	**0/5**	**0/5**			**0/5**	**0/5**		
1.600	**0/5**	**0/5**			**0/5**	**0/5**		
13-weeks NTP 2015 study								
0	10/10	10/10	194	111	10/10	10/10	69.8	79.6
25	10/10	10/10	202	114	10/10	10/10	61.5	**57.9**
50	10/10	10/10	204	117	10/10	10/10	**53.0**	**58.7**
100	10/10	10/10	190	112	10/10	10/10	**43.2**	**56.1**

200	10/10	10/10	206	116	8/10	10/10	**44.1**	**46.4**
400	10/10	10/10	190	103		6/10		**53.3**

Numbers in bold represent high differences

Microscopic lesions of the nose were noted in both sexes of rats. Effects in the nose were reported in the 13-week study at all tested concentrations (some of the following effects from 25 mg/m^3), including olfactory epithelium atrophy, mineralisation, necrosis and turbinate atrophy. These effects were of minimal severity at low doses, but the severity increased with increased concentrations for most of the effects observed. The nose was also the target organ in male and female mice in the 2-week study. Lesions in the nose included respiratory epithelial necrosis in all dosed males and in females at the highest doses. In addition, relative lung weights in the two-week study increased in males even at the lowest dose (15%), with a statistically significant (P<0.01) increase of 18% at the top dose, whereas in females a non-significant increase 7.7-11% at all doses was noted. In the 13-week study, relative lung weights remained practically unchanged in both sexes.

Effects in kidneys were also seen (renal tubules casts) at higher doses (800 mg/m^3). In rats, increased kidney weights were observed in both sexes. Clinical chemistry in the 13-week study supported the effects in kidneys. Exposure concentration-related minimal to mild increases (≤10%) were observed in total protein and globulin concentrations on days 3 and 23 in both male and female rats in various exposed groups. In addition, albumin and urea nitrogen was minimally increased during the first 25 days of the study. The total protein, albumin, globulin, and urea nitrogen concentrations returned to chamber control levels by week 14 and were consistent with possible mild dehydration, not confirmed as water consumption was not recorded.

In rats, adverse effects were described in the liver, characterised by centrilobular cytoplasmic alteration from mild to moderate severity in the two-week study from 100 mg/m^3, and from minimal to mild severity in the 13-week study from 50 mg/m^3.

In mice, effects were consistently observed in kidney, liver and nose. Critical effects were reported in the kidneys in males, with renal tubule necrosis, granular casts and renal tubule regeneration in the 2-week study from 100 mg/m^3. At this concentration no general systemic toxicity was observed. In the 13-week study, nephropathy was reported from 50 mg/m^3, which was characterised by minimal to mild tubule necrosis and cast formation, renal tubule regeneration, mild inflammatory infiltrates of lymphocytes, macrophages, and neutrophils within the interstitium and subcapsular areas, along with occasional tubule mineralisation. At this concentration, no mortality was noticed in male or female mice, but reduction in body weight gain exceeded 15% for males and 20% for females compared to controls. During the study duration, though, dosed animals continued to gain weight.

In the two NTP (2015) studies, relevant effects were also described in the nose, with necrosis of the respiratory epithelium (2-week study, in males at ≥ 200 mg/m^3) or respiratory epithelium squamous metaplasia (13-week study, in males and females at ≥ 100 mg/m^3). In all surviving groups of exposed females in the 2-week study, absolute and relative lung weights were significantly greater (up to 36%) compared to controls. In the 13-week study, gross lesions potentially related to exposure were observed in the lung of 5 female mice, including pale to white, 1 to 7 mm diameter foci. In the same study, the respiratory system as a whole appeared vulnerable, with laryngeal lesions (necrosis and respiratory epithelium hyperplasia and squamous metaplasia) being observed at early death

in 400 mg/m^3 exposed females and bronchial epithelium necrosis and histiocytic inflammation of the lung in the same group.

Liver necrosis was noted in the 2-week study from 400 mg/m^3 for both sexes (minimal for surviving animals, marked for all early-death mice). In the 13-week study minimal necrosis for females from 50 mg/m^3 to marked necrosis at 400 mg/m3 ($p<0.01$) was reported, whereas for males, mild necrosis at 200 mg/m^3 was observed.

In the table below, the main target organs recognised for each relevant study described in the CLH report, are summarised, along with the classification attributed. Regardless of the Klimisch rating of the studies, even for those of lower quality (due to technical issues, such as use of only one dose, lack of experimental details etc.), the reported findings qualitatively support the results of the NTP studies, in particular by identifying the liver and kidney as the main target organs. Details are also provided regarding the organs monitored in each study in order to assess more carefully potential effects in any other target organs than those already identified.

Table: *Summary of the main target organs following inhalation of VDC, the effective dose and the classification attributed for each relevant study described in the CLH report*

Inhalation Study	**Effective dose (respective effects)**	**Target organ toxicity relevant for classification***	**Organs monitored**	**Classification category based on the dose causing target organ toxicity relevant for classification***
* in case of multiple doses and/or multiple effects in parenthesis the minimum dose where the respective effect is observed, is mentioned				
Rats				
NTP, 2015 Klimisch 1 <u>2 weeks</u> 6 h/day, 5 days/week	100 mg/m^3 (0.1 mg/L) (Centrilobular cytoplasmic alteration in the liver)	Liver Centrilobular cytoplasmic alteration (100 mg/m^3) Kidney Renal tubule, casts (800 mg/m^3)	Organs weighed were heart, right kidney, liver, lung, right testis, and thymus. In addition to gross lesions and tissue masses, the eyes, kidney (except 50 ppm female mice), liver, lung, and nose were examined to a no-effect level.	**Category 1** (C ≤ 0.2 mg/L/6h/day) Liver: 0.017mg/L < 0.2 mg/L
NTP, 2015 Klimisch 1 <u>13 weeks</u> 6 h/day, 5	25 mg/m^3 (0.025 mg/L) (Olfactory epithelium	Nose (25 mg/m^3) Olfactory epithelium atrophy, mineralisation, necrosis	The heart, right kidney, liver, lungs, right testis, and	**Category 1** (C ≤ 0.2 mg/L/6h/day) Nose: 0.025

days/week	mineralisation/ atrophy in the nose)	and turbinate atrophy Liver Centrilobular cytoplasmic alteration/ vacuolisation (males 50 mg/m^3 L; females 200 mg/m^3)	thymus were weighed. Complete histopathologic examinations. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle,	mg/L < 0.2 mg/L

			thymus, thyroid gland, trachea, urinary bladder, and uterus.	
Rampy et al., 1977 /Quast et al, 1986 Klimisch 2 <u>18 months</u> 6 h/day, 5 days/week	300 mg/m^3 (0.3 mg/L)	Liver Hepatocellular fatty change consistent in males and females	At terminal necropsy, the brain, heart, liver, kidneys, and testes were weighed. Microscopic examinations were generally conducted on the followings organs: accessory sex glands, adipose tissue, adrenals, aorta, bone marrow (sternal), brain, epididymides, esophagus, heart, intestines (large and small), kidneys, liver, lungs, lymph nodes (mesenteric, mediastinal), mammy gland, nasal turbinates, ovaries, oviduct, pancreas, parathyroid, peripheral nerve, pituitary gland, prostate, salivary glands, skeletal muscle, skin, eye, spleen, spinal cord, sternum, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, uterus, and any gross lesion or mass.	**No classification** (C >1.0 mg/L/6h/day) Liver: 1.8 mg/L>1.0 mg/L

Lee et al., 1977 Klimisch 3 <u>12 months</u> 6 h/d, 5 days per week	220 mg/m^3 (0.22 mg/L) (only one dose tested)	Liver A mild to markedly severe focal, disseminated vacuolisation, probably fatty change	Gross examination was carefully performed on all tissues including the brain, pituitary, thyroids, respiratory tract, alimentary canal, urogenital organs, thymus, heart, liver, pancreas, spleen, mesenteric lymph nodes, and body cavities. The brain, liver, kidneys, spleen and gonads were removed and weighed. Tumors with adjacent normal tissues and the whole or portions of the various tissues were fixed, processed, sectioned, and stained for microscopic examination.	**Category 2** (0.2 < C ≤ 1.0 mg/L/6h/day) Liver: 0.2 < 0.88 mg/L < 1.0 mg/L
Maltoni et al., 1977 Maltoni et al., 1984 Klimisch 3 <u>52 weeks</u> 4 h/day, 4-5 days/week	600-800 mg/m^3 (0.6-0.8mg/L)	Liver Hepatocyte vacuolisation, cloudy swelling, fatty degeneration, necrobiosis and necrosis	Complete autopsy Histological examinations on the Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, river, kidneys, spleen, stomach, different segments of the intestine,	**No classification** (C >1.0 mg/L/6h/day) 1.6-2.1 mg/L>1.0 mg/L

			bladder, brain, bone marrow (sternum) and any other organs with pathological lesions. Cytological examinations were carried out on the bone marrow of the femur.	
Prendergast et al., 1967 Klimisch 3 <u>13 weeks</u> Continuous exposure	189 mg/m^3 (0.189 mg/L)	Liver (189 mg/m^3) Fatty metamorphosis, focal necrosis, haemosiderosis deposition, lymphocytic infiltration, bile duct proliferation and fibrosis Kidney (189 mg/m^3) Nuclear hypertrophy of the tubular epithelium	At the termination of each study, animals were sacrificed, autopsied, and sections of heart, lung, liver, spleen, and kidney were taken for histopathologic evaluation.	**Category 2** (0,2 < C ≤ 1,0 mg/L/6h/day) 0.758 mg/L<0.2 mg/L
Anonymous, 2004 <u>13 weeks</u> 6 h/day, 5 days/week	400 mg/m^3 (0.4 mg/L)	Liver Mononuclear cell aggregates and necrotic hepatocytes, hepatocellular vacuolation and hepatocellular hypertrophy	Organ weight (liver), gross examination at necropsy, microscopic examination of liver and muscle, and detailed microscopic examination of peripheral nervous system organs were evaluated.	**Category 2** (0,2 < C ≤ 1,0 mg/L/6h/day) Liver: 0,2 < 0.4 mg/L < 1.0 mg/L
Anonymous, 1979h Klimisch 4 <u>30 days</u>	-	-	Macroscopic observation, organ weight (heart, liver, kidney, spleen, testis, thyroid, adrenal gland and lung), body weight (on 20 rats) and histology on major organs (heart, aorta,	**No classification**

			trachea, lung, esophagus, stomach, small intestine, colon, parotid glandular, liver, pancreas, spleen, thymus, lymph nodes, epididymis prostate, semen bubble, ovaries, uterus, skeletal muscle, teeth, skin, eye with optic nerve) were conducted	
Gage et al., 1970 Klimisch 4 <u>4 weeks</u> 6h/day, 5 days/week	2000 mg/m^3 (2 mg/L)	Liver (2000 mg/m^3) Cell degeneration Nose (starting at 800 mg/m^3) Slight nose irritation with no significant findings noted at the autopsy, not relevant for classification alone		**Category 2** (0,2 < C ≤ 1,0 mg/L/6h/day) Liver: 0.62 mg/L<1.0 mg/L
Mice				
NTP, 2015 Klimisch 1 <u>2 weeks</u> 6 h/day, 5 days/week	100 mg/m^3 (0.1 mg/L) (Kidney effects)	Liver (males: 200 mg/m^3, females 400 mg/m^3) Hepatic necrosis Kidney (100 mg/m^3) Minimal to moderate renal tubule necrosis and granular casts Mild to moderate renal tubule regeneration Nose (from 200 mg/m^3 in males only) Minimal necrosis of the respiratory epithelium	Organs weighed were heart, right kidney, liver, lung, right testis, and thymus. Histopathology In addition to gross lesions and tissue masses, the eyes, kidney (except 50 ppm female mice), liver, lung, and nose were examined to a no-effect level.	**Category 1** (C ≤ 0.2 mg/L/6h/day) Kidney: 0.017 mg/L < 0.2 mg/L
NTP, 2015 Klimisch 1 <u>13 weeks</u> 6 h/day, 5 days/week	50 mg/m^3 (0.05 mg/L) (Minimal to moderate nephropathy in males)	Liver Necrosis (50 mg/m^3) (from individual hypereosinophilic hepatocytes with nuclear pyknosis and karyolysis to hypereosinophilic	The heart, right kidney, liver, lungs, right testis, and thymus were weighed. Complete histopathologic	**Category 1** (C ≤ 0.2 mg/litre/6h/day) Kidney: 0.05 mg/L<0.2 mg/L

		coagulum) Centrilobular hepatocyte hypertrophy (female 400 mg/m^3) Kidney Renal tubule necrosis, protein cast formation in mice that experienced early death and nephropathy in those surviving to terminal kill. Marked renal tubules necrosis (200 mg/m^3) Minimal to moderate nephropathy in males (50 mg/m^3) Nose (only females 400 mg/m^3) Minimal to moderate necrosis of the nasal respiratory epithelium and minimal turbinate atrophy Lung (only females 400 mg/m^3) Bronchial epithelium necrosis Histiocytic inflammation Larynx Necrosis and respiratory epithelium hyperplasia (400 mg/m^3) and squamous metaplasia (100 mg/m^3)	examinations In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.	

Lee et al., 1977 Klimisch 3 12 months 6 h/day, 5 days/week	220 mg/m^3 (0.22 mg/L) (only one dose tested)	Liver Enlarged and basophilic hepatocytes with enlarged nuclei, large round eosinophilic inclusions; mitotic figures or polyploidy; microfoci of mononuclear cells; focal degeneration and necrosis	Gross examination, especially for any appearance of abnormal growth or other lesions, was carefully performed on all tissues including the brain, pituitary, thyroids, respiratory tract, alimentary canal, urogenital organs, thymus, heart, liver, pancreas, spleen, mesenteric lymph nodes, and body cavities. The brain, liver, kidneys, spleen and gonads were removed and weighed. Tumors with adjacent normal tissues and the whole or portions of the various tissues were fixed, processed, sectioned, and stained for microscopic examination.	**Category 2** (0,2 < C ≤ 1,0 mg/L/6h/day) Liver: 0.2 < 0.88 mg/L < 1.0 mg/L
Maltoni et al., 1977 Maltoni et al., 1984 Klimisch 3 52 weeks	Effects observed from 40 mg/m^3 but even in controls, no statistical difference	Liver (considered not treatment related and not relevant for classification) Hepatocyte vacuolisation, cloudy swelling, fatty degeneration, necrobiosis and necrosis) and amyloidosis	A complete autopsy is carried out on each animal. Histological examinations are performed on the Zymbal glands, interscapular brown fat,	**No classification**

		Kidney (considered not treatment related and not relevant for classification) Cloudy swelling and necrosis), amyloidosis of glomeruli and chronic nephritis	salivary glands, tongue, lungs, liver, kidneys, spleen, stomach, different segments of the intestine, bladder, brain, bone marrow (sternum) and any other organs with pathological lesions. Furthermore, cytological examinations are carried out on the bone marrow of the femur.	
Hamster				
Maltoni et al., 1977 Maltoni et al., 1984 Klimisch 3 52 weeks	NOAEC=100 mg/m^3	No effects observed	A complete autopsy was carried out on each animal. Histological examinations were performed on the Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, river, kidneys, spleen, stomach, different segments of the intestine, bladder, brain, bone marrow (sternum) and any other organs with pathological lesions. Furthermore, cytological examinations were carried	**No classification**

			out on the bone marrow of the femur.	
Prendergast et al., 1967 Klimisch 3 <u>13 weeks</u> Continuous exposure	Guinea Pigs 189 mg/m^3 (0.189 mg/L)	Liver (effects of low toxicological significance) ↑SGPT ↑alkaline phosphatase	At the termination of each study, animals were sacrificed, autopsied, and sections of heart, lung, liver, spleen, and kidney were taken for histopathologic evaluation.	**No classification**
	Monkeys 189 mg/m^3 (0.189 mg/L)	Liver Fatty metamorphosis, focal necrosis, haemosiderosis deposition, lympholytic infiltration, bile duct proliferation, fibrosis	At the termination of each study, animals were sacrificed, autopsied, and sections of heart, lung, liver, spleen, and kidney as well as on sections of brain, spinal cord, and adrenal gland were taken for histopathologic evaluation.	**Category 2** (0,2 < C ≤ 1,0 mg/L/6h/day) Liver: 0.758 mg/L<0.2 mg/L
	Rabbits NOAEC = 189 mg/m^3	No effects observed	At the termination of each study, animals were sacrificed, autopsied, and sections of heart, lung, liver, spleen, and kidney were taken for histopathologic evaluation.	**No classification**
	Dogs 189 mg/m^3 (0.189 mg/L)	Liver Fatty metamorphosis, focal necrosis, haemosiderosis	At the termination of each study, animals were	**Category 2** (0,2 < C ≤ 1,0 mg/L/6h/day)

		deposition, lympholytic infiltration, bile duct proliferation, fibrosis	sacrificed, autopsied, and sections of heart, lung, liver, spleen, and kidney as well as on sections of brain, spinal cord, thyroid gland and adrenal gland were taken for histopathologic evaluation.	Liver: 0.758 mg/L<0.2 mg/L

**Comparison of the extrapolated effective dose to 90-day/6-hr exposure with the Guidance Value for each category*

In conclusion, as no human data is available, reliable data on experimental animals after repeated exposure to VDC will be used for classification purposes. As described above, inhalation appears to be the most detrimental route of exposure for VDC. The available oral studies support the classification in Category 2 at best, whereas several studies by inhalation, and particularly the most reliable ones (NTP, 2015) support classification in Category 1. The classification category of VDC should therefore be based on the studies performed via the inhalation route, according to the CLP guidance (2017).

The four studies in rats and mice performed by the NTP in 2015 (two-week and 13-week) are studies of high quality, that are used as the key studies to conclude on the classification of VDC.

The nose effects from 0.025 mg/L in rats described in the NTP (2015) 13-week study (olfactory epithelium necrosis, olfactory epithelium and turbinate atrophy) can be regarded as of significant toxicity and, along with the cluster of effects in lungs and trachea observed, do thus lead to a Category 1 classification. In the same way, renal tubule necrosis, granular casts and renal tubule regeneration (from 0.1 mg/L in the 2-week) and nephropathy (from 0.05 mg/L at the 13-week study) based on a cluster of effects observed in mice, as explained above, are also considered significant effects, which lead to classification in Category 1. Moreover, in the 2-week rat study, the critical adverse effects leading to classification were observed in the liver, occurred from 0.1 mg/L and were described as centrilobular cytoplasmic alteration from minimal to mild severity. At higher concentrations the severity increased and centrilobular necrosis was observed consistent with a more severe stage of hepatocellular damage. Liver alterations are therefore considered as significant effects clearly indicating toxicologically relevant functional disturbance of the organ. The results of the other studies of lower Klimisch rating described in the table above, confirm the target organs of VDC toxicity being the liver, kidney and respiratory tract.

The LOAEC Associated with some of these critical effects are 0.025 mg/L in the 13-week rat study and 0.05 mg/L in the 13-week mouse study. Due to the CLP Regulation guidance values for classification in category 1 via the inhalation route (C ≤ 0.2 mg/L), these 13-week studies lead to a classification of VDC in Category 1. The LOAEC for some of the critical effects in rats and mice in the 2-week studies (NTP, 2015) is 0.1 mg/L and lead consistently

to the same category of classification.

Therefore, RAC considers that classification of VDC as **STOT RE 1; H372: Causes damage to organs through prolonged or repeated exposure (respiratory tract, kidney, liver)** is warranted.

10.11 Aspiration hazard

Not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 27: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Test type: Biodegradation in water : Ready biodegradability (screening studies) Mixture of sewage, soil and natural water OECD Guideline 301 D (Ready Biodegradability: Closed Bottle Test)	Not biodegradable in water under aerobic condition % Degradation of test substance: 0 after 4 weeks	2 (reliable with restrictions) Key study experimental result Test material: vinylidene chloride (VDC)	Anonymous (1989) in the registration dossier (Japanese version); Anonymous (1992) (English version)
Test type: Biodegradation in water (screening tests) Activated sludge, domestic Static-culture flask-screening procedure of Bunch and Chambers (Jour. Water Poll. Control Fed., 39, 181 (1967)) modified.	Biodegradable under aerobic conditions with gradual adaptation from the microorganisms % Dissipation of test substance: 78% (5 mg/L) and 45 % (10 mg/L) after 7 days 100% after a subsequent subculture of 7 days (adaptation) % Volatilization in abiotic control: 24% (5mg/L) and 15% (10 mg/L) in 10 days	2 (reliable with restrictions) but not relevant experimental result Test material: vinylidene chloride (VDC)	Tabak et *al.* (1981)
Test type: Anaerobic biodegradation in water (screening tests) Cultures isolated from sediment in mineral medium Biodegradation of VDC by live culture of methane-utilizing bacteria and control with killed culture	Readily biotransformation in test condition (anaerobic) % Dissipation of test substance: 70% after 48 hours % Volatilization in abiotic control: 30% after 48 hours	2 (reliable with restrictions) but not relevant experimental result Test material: vinylidene chloride (VDC)	Fogel et *al.* (1986)

Method	Results	Remarks	Reference
Test type: Biodegradation in anaerobic water and sediment: (simulation testing) Mixture of sewage, soil and natural water Microcosms constructed with authentic aquifer material that receives municipal landfill leachate and is known to support methanogenesis	Significant anaerobic degradation after a long lag time (16 weeks) % Dissipation of test substance: 0% after 7 weeks 52% after 16 weeks 60-100 % after 40 weeks % Volatilization in abiotic control: No data after 7 weeks 23% after 16 weeks 48% after 40 weeks	2 (reliable with restrictions) but not relevant Key study experimental result Test material: vinylidene chloride (VDC)	Wilson B.H. et *al.* (1986)

11.1.1 Ready biodegradability

One study performed according to the OECD TG 301 D (Ready Biodegradability: Closed Bottle Test) (Anonymous, 1989 and 1992) is available. The details on the results of this study are not available to the DS and GLP was not mentioned. However, the study was generated by the Japanese Competent Authorities and followed a standard guideline suitable for a volatile substance, thus it is considered reliable with restrictions (Reliability 2) and a key study. The test was conducted in aerobic conditions with a mixture of sewage, soil and natural water for 4 weeks. The reference substance was aniline. Initial concentration of VDC was 9.7 mg/L. The study showed no ultimate biodegradation of vinylidene chloride (VDC) (0%) based on O_2 consumption after 28 days of incubation with activated sludge. The level of aniline determined from the BOD (biochemical oxygen demand) was 73% at 28 days after the start of the test, thus confirming that the test conditions were valid.

Quantitative estimation method (QSAR) for estimating the degree of biodegradability of organic substances may be used to predict that a substance is not rapidly degradable, or be used in a weight of evidence approach (ECHA, 2017).

The DS made estimations using BIOWIN (v4.10) models 1, 2, 5 & 6 to calculate the probability score that a substance under aerobic conditions with mixed cultures of microorganisms will be rapidly or ready biodegradable in the environment, according to CLP guidance (ECHA, 2017). The results for these 4 models are < 0.5 and the substance should be regarded as not rapidly degradable (0.4786 for BIOWIN 1 and 0.117 for BIOWIN 2) and not ready biodegradable (0.4383 for BIOWIN 5 and 0.1833 for BIOWIN 6). Vinylidene chloride (VDC) having a molecular weight (MW) of 96.94 g/mol, it is included in the MW range (31-698) of the training set compounds. Thus, the results are considered to be in the applicability domain of the models.

11.1.2 BOD_5/COD

Not assessed in this dossier.

11.1.3 Hydrolysis

Hydrolysis rate constant for vinylidene chloride (VDC) has been measured in dilute aqueous solutions and Arrhenius parameters were determined for both neutral and alkaline hydrolysis reactions (Jeffers, 1989). This investigation does not follow the standard guideline recommendations, thus it is considered reliable with

restrictions. The half-life calculated is $1.2x10^8$ years in neutral to slightly basic pH for this study, indicating that the substance is stable in water, and one product was identified from alkaline hydrolysis: chloroacetylene. Other publications can also be found showing a faster rate of degradation. Cline and Delfino (1987) determined an half-life of 6 to 9 months for the same substance (pH range from 4.5 to 8.5) and Schmidt-Bleek et *al*. (1982) estimated a DT_{50} of 2 years (pH 7). Taken together, these results show that hydrolysis is not a significant degradation pathway for vinylidene chloride (VDC). Moreover, following the Guidance on the Application of the CLP Criteria (July 2017), data on hydrolysis might be considered for classification purposes only when the longest half-life t½ determined within the pH range 4-9 is shorter than 16 days..

11.1.4 Other convincing scientific evidence

Not assessed in this dossier.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Not assessed in this dossier.

11.1.4.2 Inherent and enhanced ready biodegradability tests

Not assessed in this dossier.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Three simulation studies were mentioned in the registration dossier. However, according to the Guidance on the Application of the CLP Criteria (July 2017), these studies are not considered relevant for classification and labelling because they refer to the use of waster water, simulate the conditions in a sewage treatment plant (STP) and /or regard anaerobic degradation.

A biodegradability study with a domestic waste water inoculum (Tabak *et al.*, 1981) showed, by gas chromatographic (GC) analysis and total organic carbon (TOC) determination, a loss of 78% and 45% of vinylidene chloride (VDC) at initial concentrations of 5 and 10 mg/L respectively in 7 days. After a subsequent subculture of 7 days (i.e. adaptation from the microorganisms), there was a loss of 100 % of VDC. Abiotic control showed that volatilization took place at a level of 15% (10 mg/L) and 24% (5mg/L) in 10 days. This study shows an important primary degradation of VDC in aerobic conditions. However, it is not possible to conclude on the ultimate biodegradation of the substance from this study. The study with VDC was conducted as part of a test battery in which the biodegradability of 114 industrial chemicals was determined and no specific information is provided on the methods used for the test with VDC. The study is not conducted according to GLP or standard guidelines, however it is considered as scientifically sound and reliable with restrictions. However, as mentioned above, this study is not considered relevant for the classification of VDC. Indeed, the use of wastewater as microbial inoculum may increase the biodegradation potential, with the presence of more suitable or adapted micro-organisms, compared to natural aquatic environments.

The study by Fogel *et al.* (1986) shows that biodegradation of VDC by methanotrophic bacteria can occur under anaerobic conditions (around 70% of loss of substance) following an incubation in sealed culture bottles for 48 hours. The study is not conducted according to GLP or standard guidelines, nevertheless it is considered as scientifically sound and reliable with restrictions. Although this study gives interesting information on the fate of the substance, according to the Guidance on the Application of the CLP Criteria (July 2017), data regarding anaerobic degradation cannot be used in relation to deciding whether a substance should be regarded as rapidly degradable. The aquatic environment is generally regarded as the aerobic compartment where the aquatic organisms, such as those employed for aquatic hazard classification, live.

Finally, one well detailed water-sediment study in anaerobics conditions (Wilson *et al.*, 1986) examined the behaviour of commonly occurring contaminants in microcosms constructed with authentic aquifer material

that receives municipal landfill leachate and is known to support methanogenesis. The study is not conducted according to GLP or standard guidelines, nevertheless it is considered as scientifically sound and reliable with restrictions. The disappearance of VDC was not rapid and a lag period of 16 weeks was required before significant degradation occurred compared to autoclaved controls. After 40 weeks, there was a decrease of 60 % to 100 % of the concentration of VDC compared with autoclaved control. There was a decrease of 80 % to 100 % of the concentration of VDC compared to initial concentration. According to the Guidance on the Application of the CLP Criteria (July 2017), results from tests simulating the conditions in a sewage treatment plant (STP) cannot be used for assessing the degradation in the aquatic environment. The microbial biomass in a STP is significantly different from the biomass in the environment, there is a considerably different composition of substrates, and the presence of rapidly mineralised organic matter in waste water may facilitate degradation of the test substance by co-metabolism. At last, as seen above, anaerobic degradation tests do not qualify either because of the specificity of the anaerobic compartments.

11.1.4.4 Photochemical degradation

Not assessed in this dossier.

11.2 Environmental fate and other relevant information

No other relevant information

11.3 Bioaccumulation

Table 28: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Test type: Bioaccumulation aquatic/sediment Common carp (Cyprinus carpio) under flow-through conditions for 6 weeks OECD Guideline 305 C (Bioaccumulation: Test for the Degree of Bioconcentration in Fish)	BCF (aquatic species): bioconcentration factors ranged from 2.5-6.4 and <13 for 500 and 50 µg/L respectively	2 (reliable with restrictions) Key study experimental result Test material: vinylidene chloride (VDC)	Anonymous(1991) in the registration dossier (Japanese version); Anonymous (1992) (English version)

11.3.1 Estimated bioaccumulation

11.3.2 Not assessed in this dossier.Measured partition coefficient and bioaccumulation test data

Three different references mentioned a measured partition coefficient value for the substance VDC. In the Verschueren Handbook of Environmental data on Organic Chemicals (1996), the log Pow value of 2.02 was specified as an experimental result. In the Howard Handbook of Environmental Fate and Exposure Data for Organic Chemicals (1989), the log octanol/water partition coefficient is 2.13. Finally, in the Lide CRC Handbook of Chemistry and Physics (2000), the log Pow of VDC is also 2.13.

Except for the calculated estimate provided in the Verschueren Handbook of Environmental data on Organic Chemicals (1996), all log Pow values provided by the reliable sources where in a narrow range, i.e. between 2.02 to 2.13. As a conservative approach, a log Pow of 2.13 is defined as the key parameter. Therefore the substance VDC does not show a real potential to bioconcentrate.

One study performed according to the OECD test guideline 305 (Anonymous, 1991) is available. This study is in Japanese and thus could not be reviewed by the DS. However, an English version without full details (Anonymous, 1992) was obtained from the lead registrant. The study was generated by the Japanese

Competent Authorities and followed a standard guideline suitable for a volatile substance, thus it is considered reliable with restrictions and a key study. The bioconcentration of VDC was studied in common carp (Cyprinus carpio) under flow-through conditions for 6 weeks. The measured VDC concentrations in the test solutions were maintained on a constant level throughout the test (486 – 493 µg/L and 46.8 – 47.8 µg/L, respectively for the two exposure groups). The bioconcentration factors were 2.5–6.4 (500 µg/L) and <13 (50 µg/L) during the 6 weeks exposure period.

Considering the log Pow of 2.13 and the measured BCF in the OECD 305 study <13, it is therefore concluded that VDC has a low potential for bioaccumulation.

11.4 Acute aquatic hazard

Table 29: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
13 days Flow through EPA-660/3-75-009	Fathead minnows *(Pimephales promelas)*	vinylidene chloride (VDC) purity >99.5%	LC50 (96h) 108 (95 % CI: 85 – 117) mg/L LC50 (7 to 13 days) 29 (95 % CI: 25 – 34) mg/L measured	2 (reliable with restrictions, adult fish, lack of information about pH and O_2) Key study experimental result	Anonymous (1977) in the registration dossier but also mentioned in Dill *et al.* (1980)
96 hours Static test conditions EPA-660/375-009	Bluegill sunfish *(Lepomis macrochirus)*	vinylidene chloride (VDC) >80%	LC50 (96 h) 74 (95 % CI: 57 – 91) mg/L nominal	3 (not reliable, static, nominal, low oxygen level) Supporting study experimental result	Buccafusco *et al.* (1981)
96 hours Static test No specific guideline	Bluegill sunfish *(Lepomis macrochirus)*	vinylidene chloride (VDC) No information on purity	LC50 (96 h) 220 mg/L nominal	3 (not reliable, adult, static, nominal, not covered, lack of information about pH and O_2) Supporting study experimental result	Dawson, G.W. (1977)
96 hours Static test No specific guideline	Tidewater silversides *(Menidia beryllina)*	vinylidene chloride (VDC) No information on purity	LC50 (96 h) 250 mg/L nominal	3 (not reliable adult, static, nominal, not covered, lack of information about pH and O_2) Supporting study experimental result	Dawson, G.W. (1977)
96 hours Static test EPA-660/375-009	Sheepshead minnows *(Cyprinodon variegatus)*	vinylidene chloride (VDC) >80%	LC50 (96 h) 250 (95 % CI: 200 – 340) mg/L nominal	3 (not reliable, static, nominal lack of information about pH and O_2) Supporting study experimental result	Heitmuller *et al.* (1981)

Method	Species	Test material	Results	Remarks	Reference
OECD 202 static test GLP study	*Daphnia Magna*	vinylidene chloride (VDC) purity 99.95%	EC50 (48h) 37 mg/L measured	1 (fully reliable, measured, covered) Key study experimental result	Anonymous (2010)
48 hours Static test EPA-660/375-009	*Daphnia Magna*	vinylidene chloride (VDC) purity 99.5%	LC50 (48 h) 11.6 (95 % CI: 9 – 14) mg/L nominal	3 (not reliable, nominal, not covered, lack of information about pH and O2) Supporting study experimental result	Dill *et al.* (1980)
48 hours Static test EPA-660/375-009	*Daphnia Magna*	vinylidene chloride (VDC) purity >80%	LC50 (48 h) 79 (95 % CI: 62 – 110) mg/L nominal	3 (not reliable, nominal, lack of information on the tested concentrations and number of replicats) Supporting study experimental result	Leblanc, G.A. (1980)
72 hours Static test No specific guideline-adaptation for volatile compound	*Chlamydomonas reinhardtii*	vinylidene chloride (VDC) purity >99%	EC50 (72 h) 9.12 (95 % CI: 7.42 – 11.3) mg/L measured	2 (reliable with restrictions, no specific guideline, lack of information about pH) Key study experimental result	Brack *et al.* (1994)
96 hours Static test Guideline of the Federal Environmental Agency (Umweltbundesamt)	*Scenedesmus subspicatus*	vinylidene chloride (VDC) purity >99%	EC50 (96 h) 410 mg/L nominal	3 (not reliable, nominal, open system, lack of information about pH) supporting study experimental result	Geyer *et al.* (1985)

11.4.1 Acute (short-term) toxicity to fish

The study on *Pimephales promelas* (Anonymous, 1977; Dill et *al*., 1980), has been identified as the key study. The acute toxicity of VDC was assessed in adult *Pimephales promelas* using a flow-through system suitable for volatile compounds. This study was conducted in accordance with the EPA guideline 'Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians' EPA-660/3-75-009 (1975). A clear plastic cover was placed over each exposure aquarium to retard volatilization of title material. The VDC

concentration was measured once a day per concentration during the study using a gas chromatograph with a flame ionization detector (GC-FID) method. No information about pH and oxygen level during experimentation was found. In this study, a LC_{50} of 107.9 mg/L (95% confidence interval: 84.6-117.4) was determined after 96 hours.

Two other studies were identified assessing the toxicity of VDC in freshwater fish. The study of Buccafusco *et al.* (1981) was conducted on young of the year (0.32-1.2 g) *Lepomis macrochirus* in a static test performed according to US EPA Guideline EPA-660/3-75-009 (1975). Since VDC is volatile, closed test systems were used but no verification of the test concentration was performed. The study was conducted as part of a test battery with several (64) other industrial chemicals and no information is provided on the methods and specific conditions for the test with VDC. In addition, the O_2 concentration, when considering all the tested chemicals, was reported to be as low as 0.3 mg/L at the end of experiments. Therefore the data were considered not reliable. In this study, the LC_{50} (24 and 96 hours) was determined to be 74 mg/L. The study of Dawson *et al.* (1977) was also conducted on adult *Lepomis macrochirus* in a static test system without following a specific guideline. Aeration of water was carried out and results were expressed in terms of nominal concentrations. Test tanks were not capped and volatilization, as described in the study, should have occurred. The procedure is considered unsuitable for a volatile compound. No data on pH, dissolved oxygen concentration and test substance concentration during the test were reported. The LC_{50} (96 h) in this study was 220 mg/L.

Additionally, two studies were identified assessing the toxicity of VDC on saltwater fish. The study of Dawson *et al*. (1977) was conducted on adult *Menidia beryllina* in a static test system without following a specific guideline. Aeration of water was carried out and results were expressed in terms of nominal concentrations. The procedure is considered unsuitable for a volatile compound. No data on pH, dissolved oxygen concentration and test substance concentration during the test were reported. The LC_{50} (96 hours) in this study was 250 mg/L. The study of Heitmuller *et al*. (1981) was conducted on juvenile (14-28 days post-hatch) *Cyprinodon variegatus* in a static test performed according to US EPA Guideline EPA-660/3-75-009. Results were expressed in terms of nominal concentrations. No verification of the test concentration was performed and it is not specified if the test containers were covered during the experimentation. The dissolved oxygen concentrations and pH were measured but not reported. Therefore the data were considered not reliable. The LC_{50} (96 hours) in this study was 250 mg/L, the NOEC (96 hours) was 80 mg/L.

11.4.2 Acute (short-term) toxicity to aquatic invertebrates

Three studies assessed the toxicity of vinylidene chloride (VDC) on the aquatic invertebrate *Daphnia magna*. But only one was assigned with a Klimisch score scientifically acceptable (1 or 2).

The key study (Anonymous, 2010) follows the OECD TG 202 and is conducted under GLP criteria. The VDC concentration was measured at test initiation and at the end of the static test using a validated GC-MS method. The study was performed using glass flasks stoppered with PTFE bungs and sealed with aluminium caps in order to avoid the loss of the test item. The test flasks were totally filled allowing no head space. Dissolved oxygen (range from 8.8 mg/L initial and 8.3 mg/L final) and pH (range from 8.03 initial and 7.75 final) were measured at the beginning and at the end of the experiment. In this study, a LC_{50} (48 hours) of 37 mg/L was determined. This result is in line with that of other, less reliable acute studies.

The DS found an additional publication that was not mentioned in the registration dossier. Dill *et al.* (1980) performed a 48 hours static test study according to US EPA Guideline EPA-660/3-75-009 (1975). It is not mentioned if the test systems were closed and no verification of the test concentration was performed. The 24 hours and 48 hours LC_{50} values are identical, and the authors suggest that it is an indication that the compound probably had volatilized from the exposure beakers. In the lack of measured concentrations and

information regarding the covering of the system, the results were considered not reliable. The LC_{50} (48 hours) was determined to be 11.6 (95 % CI: 9 – 14) mg/L.

The study of Leblanc (1980) was also conducted in a static test system and results were expressed in terms of nominal concentrations. The study was conducted as part of a test battery with several other industrial chemicals and no information is provided on the methods and specific conditions for the test with VDC. Closed test systems were used, however no indication is given on the tested concentrations and no verification of the test concentrations was performed. Information found in the publication suggests that only one replicate (with 15 daphnids) was used for each concentration tested. Therefore the data were considered not reliable. The LC_{50} (48 hours) in this study was 79 (95 % CI: 62 – 110) mg/L.

11.4.3 Acute (short-term) toxicity to algae or other aquatic plants

Two studies were selected to assess the toxicity of vinylidene chloride (VDC) on aquatic algae.

The study of Brack and Rottler (1994) was conducted on freshwater living species *Chlamydomonas reinhardtii*, with an exposure duration of 72 hours. A static closed system with a $KHCO_3/K_2CO_3$ buffer to ensure CO_2 supply was used to investigate the toxicity of VDC. The study does not follow a specific guideline, however it is scientifically sound and well documented. Concentrations were measured and the assay is appropriate for organic volatile compounds. The study was conducted as part of a test battery with several other industrial chemicals and the pH in the medium ranged from 6.5 to 7.5 for all the chemicals tested, although no data on pH was clearly specified during the experiment with vinylidene chloride (VDC). The result EC_{50} (72 hours) of 9.12 mg/L (95 % CI: 7.42 – 11.3 mg/L) based on biomass growth inhibition was determined in this study. The study was considered reliable with restrictions.

The study of Geyer *et al.* (1985) was conducted according to the test guideline of the Federal Environmental Agency (Umweltbundesamt) on freshwater living species *Scenedesmus subspicatus*. The test flasks were closed, however Kapsenberg caps used in this study allow gaz exchange with the environment. Thus the test system is not appropriate for volatile compounds. The concentrations were not measured and reported values are probably overestimated. Moreover, information on pH variation during testing are not provided. The result obtained in this study, based on biomass growth inhibition, is EC_{50} of 410 mg/L with an exposure duration of 96 hours.

11.4.4 Acute (short-term) toxicity to other aquatic organisms

Not assessed in this dossier.

11.5 Long-term aquatic hazard

Table 30: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
72 hours Static test No specific guideline-adaptation for volatile compound	*Chlamydomonas reinhardtii*	vinylidene chloride (VDC) purity >99%	EC10 (72 h) 3.94 (95 % CI: 2.44 – 5.15) mg/L measured	2 (reliable with restrictions, no specific guideline, lack of information about pH) Key study experimental result	Brack *et al.* (1994)
96 hours Static test Guideline of the Federal	*Scenedesmus subspicatus*	vinylidene chloride (VDC) purity >99%	EC10 (96 h) 240 mg/L nominal	3 (not reliable, nominal, open system, lack of information about pH)	Geyer *et al.* (1985)

Method	Species	Test material	Results	Remarks	Reference
Environmental Agency (Umweltbundesamt)				supporting study experimental result	

11.5.1 Chronic toxicity to fish

No other relevant information

11.5.2 Chronic toxicity to aquatic invertebrates

No other relevant information

11.5.3 Chronic toxicity to algae or other aquatic plants

Two studies were selected to assess the toxicity of VDC on aquatic algae. These two studies are also described in the section 11.4.3 "acute (short-term) toxicity to algae or other aquatic plants studies" above.

The study of Brack and Rottler (1994) was conducted on freshwater living species *Chlamydomonas reinhardtii*, with an exposure duration of 72 hours in a static test system. The result EC_{10} of 3.94 mg/L is well documented and scientifically acceptable.

The study of Geyer *et al.* (1985) was conducted on freshwater living species *Scenedesmus subspicatus* with an exposure duration of 96 hours and resulted in an EC_{10} of 240 mg/L. However, the open test system is not appropriate for volatile compound and reported values are probably higher than the real one.

11.5.4 Chronic toxicity to other aquatic organisms

Not assessed in this dossier.

11.6 Comparison with the CLP criteria

11.6.1 Acute aquatic hazard

One acceptable study is available for each category of aquatic organisms. In the fish study, Dill *et al.* (1980) concluded in a LC_{50} (96 hours) of 108 mg/L. The study on *Daphnia Magna* (Anonymous, 2010) states the EC_{50} to be 37 mg/L. Finally, Brack and Rottler (1994) demonstrated a EC_{50} (72 hours) in *Chlamydomonas reinhardtii* of 9.12 mg/L. The lowest endpoint is the value of 9.12 mg/L for algae and it does not fulfill the criteria for an aquatic acute classification under the CLP regulation.

	Criteria for acute environmental hazards	vinylidene chloride	Conclusion
Acute Aquatic Toxicity	Cat. 1: LC50/EC50/ErC50 ≤ 1 mg/L	Fish: 96h-LC50= 108 mg/L (*Pimephales promelas*) Invertebrates: 48h-EC50= 37 mg/L (*Daphnia magna*) Algae: 72h-EC50= 9.12 mg/L (*Chlamydomonas reinhardtii*)	**No classification required**

11.6.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Based on the ready biodegradability test, and the supported evidence of the hydrolysis studies and QSAR biowins models, VDC is considered as not rapidly degradable.

Collected information supports the low potential for bioaccumulation of vinylidene chloride (VDC) (Log Pow < 4 and BCF < 500).

There is no reliable data for aquatic chronic toxicity for fish and invertebrates. One reliable study is available for chronic toxicity to algae with an EC_{10} value of 3.94 mg/L which does not lead to chronic classification. However, in the case where only one trophic level with adequate chronic toxicity data is available, an assessment is made according to the criteria given in Table 4.1.0(b)(i) or 4.1.0(b)(ii) (depending on information on rapid degradation and/or bioaccumulation), and (if for the other trophic level(s) adequate acute toxicity data are available) according to the criteria given in Table 4.1.0(b)(iii) (Guidance on the Application of the CLP Criteria, July 2017). Then, the classification is made according to the most stringent outcome. In this case, considering the acute data on toxicity for *Daphnia magna* with an EC_{50} of 37 mg/L, a 48 hr EC_{50} (for crustacea) within 10 to 100 mg/L leads to chronic 3 category for hazardous to the aquatic environment. Therefore, the substance needs to be classified H412 for aquatic chronic hazards.

	Criteria for long-term environmental hazards	**vinylidene chloride**	**Conclusion**
Rapid degradation	Half-life hydrolysis < 16 days	$1.2x10^8$ years at 25 °C and pH 7 (Jeffers, 1989)	**Not rapidly degradable**
	Readily biodegradable in a 28-day test for ready biodegradability (> 70 % DOC removal or > 60 % theoretical oxygen demand, theoretical carbon dioxide)	0% BOD in 28-day (Anonymous, 1989)	
	Primary degradation: half-life < 16 days (if degradation products do not fulfil criteria for classification as hazardous to the aquatic environment)	No relevant data available	
Bioaccumulation	BCF ≥ 500	BCF ≤ 2.5 - 13	**Not bioaccumulative** (low potential for bioconcentration in the aquatic environment)
Chronic Aquatic Toxicity	Not rapidly degradable substances: Cat. 1: NOEC ≤ 0.1 mg/L Cat. 2: NOEC ≤ 1 mg/L (based on Table 4.1.0 (b) (i) of the CLP Regulation)	Algae: 72h-EC10= 3.94 mg/L (*Chlamydomonas reinhardtii*)	No classification required
	Surrogate approach in absence of appropriate chronic toxicity reference data (based on Table 4.1.0 (b) (iii) of the CLP Regulation): Not rapidly degradable substances and/or bioaccumulative substances: Cat. 1: E/LC50 ≤ 1 mg/L Cat. 2: E/LC50 > 1 to ≤ 10 mg/L Cat. 3: E/LC50 > 10 to ≤ 100 mg/L	Fish: 96h-LC50= 108 mg/L (*Pimephales promelas*) Invertebrates: 48h-EC50= 37 mg/L (*Daphnia magna*)	**Aquatic Chronic 3** (based on invertebrate-EC50)

11.7 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Based on the CLP regulation, the substance does not need to be classified for aquatic acute hazards.

Based on the CLP regulation, **the substance needs to be classified H412 for aquatic chronic hazards** according to the criteria given in Table 4.1.0(b)(iii) and considering the acute data on toxicity for *Daphnia magna* EC_{50} 37 mg/L, (48 hr EC_{50} (for crustacea) within 10 to 100 mg/L) corresponding to chronic 3 category for hazardous to the aquatic environment for a substance not rapidly degradable.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The substance is included in Annex VI of the CLP Regulation without an aquatic hazard classification and is very volatile (vapour pressure of 66340 Pa at 20°C). The Dossier Submitter (DS) proposed to classify 1,1-dichloroethylene with Aquatic Chronic 3. The acute L/EC_{50} values were all above the cut-off of 1 mg/L for aquatic acute classification and acute classification was not warranted. The only available chronic toxicity data EC_{10} for algae was above the classification cut-off value of 1 mg/L for a not rapidly degradable substance. The surrogate system with fish LC_{50} of 107.9 mg/L did not warrant classification. Whereas the EC_{50} of 37 mg/L for *Daphnia* for a not rapidly degradable substance warranted Aquatic Chronic 3 classification.

Degradation

There was one reliable ready biodegradability study (OECD TG 301 D) available showing 0% degradation after 4 weeks. Estimations using BIOWIN (v.4.10) models also predicted the substance being not rapidly degradable.

The DS concluded that hydrolysis is not a significant degradation pathway for 1,1-dichloroethylene. In a study by Jeffers (1989) half-life of 1.2 x 10^8 years in neutral to slightly basic pH was calculated. Chloroacetylene was identified as a hydrolysis product in alkaline hydrolysis. The study did not follow standard guideline recommendations but was considered reliable with restrictions. Cline and Delfino (1987) determined half-life of 6 to 9 months (pH range from 4.5 to 8.5) and Schmidt-Bleek et *al*. (1982) estimated a DT_{50} of 2 years (pH 7).

There were no relevant water/sediment or simulation studies available.

The DS concluded that 1,1-dichloroethylene is not rapidly degradable.

Bioaccumulation

There was one reliable fish bioaccumulation study (OECD TG 305 C (1981), GLP) available for the substance. The test was suitable for volatile substances and the test substance concentrations were maintained on a constant level. Test was performed with *Cyprinus carpio* under flow-through conditions for 6 weeks. BCF values ranged from 2.5 to 6.4 and <13 at concentrations 500 and 50 µg/L, respectively.

Log Pow values from reliable sources ranged from 2.02 to 2.13 showing low potential for bioaccumulation.

The DS concluded that 1,1-dichloroethylene has a low potential for bioaccumulation.

Acute aquatic toxicity

Table: *Summary of reliable information on acute aquatic toxicity*

Method (*	Species	Test material purity	Results	Remarks	Reference
Fish					
EPA-660/3-75-009 13 days flow-through	Fathead minnow *(Pimephales promelas)*	>99.5%	96-h LC_{50} 108 mg/L measured	2 (reliable with restrictions) Clear plastic cover over each aquarium	Anonymous (1977)
Invertebrates					
OECD 202, GLP static test	*Daphnia magna*	99.95%	48-h EC_{50} 37 mg/L measured	1 (reliable) Glass flasks stoppered with PTFE bund and sealed with aluminium caps, no head space	Anonymous (2010)
Algae					
No specific guideline **72-hour static test**	*Chlamydomonas reinhardtii*	>99%	72-hr EC_{50} 9.12 mg/L measured biomass growth inhibition	2 (reliable with restrictions) adaptation for volatile compound	Brack *et al*. (1994)

(* Vapour pressure of the substance is 66340 Pa at 20°C

There was one reliable study available for each trophic level. The results were a 96-hour LC_{50} of 108 mg/L for fish, a 48-hour EC_{50} of 37 mg/L for *Daphnia magna* and a 72-hour EC_{50} of 9.12 mg/L for *Chlamydomonas reinhardtii*.

The DS also presented supporting studies that were, however, considered not reliable.

The lowest acute aquatic toxicity value was a 72-hour EC_{50} of 9.12 mg/L.

Chronic aquatic toxicity

T*able:* *Summary of reliable information on chronic aquatic toxicity*

Method (*	Species	Test material purity	Results	Remarks	Reference
No specific guideline	*Chlamydomonas reinhardtii*	>99%	72-hr EC_{10} 3.94	2 (reliable with	Brack *et al.* (1994)

72-hr static test			mg/L measured	restrictions) adaptation for volatile compound	

(* Vapour pressure of the substance is 66340 Pa at 20°C

There was only one reliable study to assess chronic toxicity of 1,1-dichloroethylene. The EC_{10} for the algae *Chlamydomonas reinhardtii* was 3.94 mg/L.

The DS also presented a supporting study considered as not reliable.

There was no data available for fish and invertebrates.

The DS concluded that no chronic aquatic classification is warranted based on the algae study because the 72-hour EC_{10} of 3.94 mg/L is above the chronic classification cut-off 1 mg/L for not rapidly degradable substances (CLP Annex I Table 4.1.0 (b) (i)).

Regarding fish and invertebrates with no chronic toxicity data, according to the DS, the surrogate system for the lowest acute toxicity result, 48-hour EC_{50} of 37 mg/L for *Daphnia magna*, warrants **Aquatic Chronic 3** classification (CLP Table I Table 4.1.0 (b)(iii)).

The *Daphnia magna* study (Anonymous, 2010) follows the OECD TG 202 (GLP) and was conducted at nominal concentrations of 0, 25, 32.8, 43.2, 57.4, 76 and 100 mg/L. The study was performed using glass flasks stoppered with PTFE bungs and sealed with aluminium caps in order to avoid the loss of the test item. The test flasks were totally filled allowing no head space. The test concentrations were measured at test initiation and at the end of the static test using a validated GC-MS method. The EC_{50} values were determined based on geometric average values of initial and final measured concentrations being 0 (< LOD), 16.0, 20.5, 29.4, 36.8, 49.3 and 70.9 mg/L. In this study a LC_{50} (48 hours) of 37 mg/L was determined.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS conclusion to consider 1,1—dichloroethylene as not rapidly degradable based on:

- no degradation in a ready biodegradability study (OECD TG 301 D) in 4 weeks
- stable to hydrolysis:
    - half-lives:
        - 1.2×10^8 years in neutral to slightly basic pH
        - 6 to 9 months (pH range from 4.5 to 8.5)
        - 2 years (pH 7)
- no relevant water/sediment or simulation studies available.

Bioaccumulation

RAC agrees with the DS conclusion to consider 1,1-dichloroethylene having a low potential for bioaccumulation based on:

- BCF for fish below the classification cut-off 500:
  - BCF from 2.5 to 6.4 and <13 at concentrations 500 and 50 µg/L, respectively
- log Pow below the classification cut-off 4:
  - log Pow 2.02 – 2.13

Aquatic toxicity

Acute

RAC agrees with the DS conclusion that **aquatic acute classification is not warranted for 1,1-dichloroethylene**. The L(E)C_{50} values for fish, invertebrates and algae were above the classification cut-off 1 mg/L (CLP Annex I Table 4.1.0 (a)).

Chronic

There is chronic toxicity data available only for algae. The 72-hour EC_{10} of 3.94 mg/L does not warrant classification (CLP Annex I Table 4.1.0 (b) (i)). There is no chronic toxicity data available on fish and Daphnia. The surrogate system for fish 96-hour LC50 of 108 mg/L does not warrant classification.

Consequently, RAC agrees with the DS to base the chronic classification on the acute *Daphnia magna* test result 48-hour EC_{50} of 37 mg/L which **warrants classification as Aquatic Chronic 3** for a not rapidly degradable substance (CLP Table I Table 4.1.0 (b)(iii)).

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not assessed in this dossier

13 ADDITIONAL LABELLING

Not assessed in this dossier

REFERENCES

Anderson D, Hodge M.C.E., Purchase I.F.H. 1977: Dominant Lethal Studies with the Halogenated Olefins Vinyl Chloride and Vinylidene Dichloride in Male CD-1 Mice. Environmental Health Perspectives (1977), 21, 71-78.

Baden J. M., Kelley M., Simmon V. F., Rice S. A. And Mazze R. I. 1978: Fluroxene Mutagenicity. Mutation Research, 58 183-191.

Baden, J.M., Kelley, M., And Mazze, R.I. 1982: Mutagenicity of experimental inhalational anesthetic agents: sevofluorane, synthane, dioxychlorane and dioxyfluorane. Anesthesiology, 56: 462-463.

Ban M,; Hettich D., Huguet N. and Cavelier L. 1995: Neurotoxicity mechanism of 1,1-dichloroethylene in mice. Toxicology Letters, 78:87-92.

Bartsch H., Malaveille C., Montesano R., Tomatis L. 1975: Tissue-mediated mutagenicity of vinylidene chloride and 2-chlorobutadiene in Salmonella typhimurium. Nature, 255(5510):641-643.

Brack W. and Rottler H. 1994: Toxicity Testing of Highly Volatile Chemicals with Green Algae. Environmental Science and Pollution Research, 1(4):223-228.

Bronzetti, G., Bauer, C., Corsi, C., Leporini, C., Nieri, R., & Del Carratore, R. 1981: Genetic activity of vinylidene chloride in yeast. Mutat. Res., 89: 179-185.

Buccafusco R.J., Ells S.J. and LeBlanc G.A. 1981: Acute Toxicity of Priority Pollutants to Bluegill (Lepomis macrochirus). Bull. Environm. Contam. Toxicol. 26:446-452.

Cline PV, Delfino JJ. 1987: American Chemical Society, Division of Environmental Chemistry Preprint, New Orleans (LA). 27:577–579 [cited in HSDB 2005].

Costa A.K. and Ivanetich K.M. 1984: Chlorinated ethylenes: their metabolism and effect on DNA repair in rat hepatocytes. Carcinogenesis, 5(12):1629-1636.

Cotti G., Maltoni C., And Giuseppe L. 1988: Long-Term Carcinogenicity Bioassay on Vinylidene Chloride Administered by Inhalation to Sprague-Dawley Rats. New Results. Annals of the New York Academy of Sciences 534(1), pp. 160-168.

Crebelli R., Andreoli C., Carere A., Conti G., Conti L., Cotta Ramusino M. and Benigni R. 1992: The induction of mitotic chromosome malsegregation in Aspergillus nidulans. Quantitative structure activity relationship (QSAR) analysis with chlorinated aliphatic hydrocarbons. Mutation Research, 266:117-134.

Dawson G.W. 1977: The acute toxicity of 47 industrials chemicals to fresh and saltwater fishes. Journal of Hazardous Materials, 1 1975-1977):303-318.

Dill D.C., McCarty, W.M, Alexander, H.C., Bartlett, E.A. 1980: Toxicity of 1,1-dichloroethylene (Vinylidene chloride) to aquatic organisms. Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, Minnesota. Report date: July 1980

Drevon C. and Kuroki T. 1979: Mutagenicity Of Vinyl Chloride, Vinylidene Chloride And Chloroprene In V79 Chinese Hamster Cells. Mutation Research, 67 173-182

ECHA, European Chemicals Agency 2017: Guidance on the Application of the CLP Criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0 - July 2017. Helsinki, Finland. https://echa.europa.eu/documents/10162/2324906/clp_en.pdf/

ECHA : Registration dossier for vinylidene chloride (2021) : https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15622

Fogel & al. 1986: Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture. Appl Environ Microbial 51:720-724.

Foureman P., Mason J.M., Valencia R., Zimmering S. 1994: Chemical Mutagenesis Testing in Drosophila.

IX. Results of 50 Coded Compounds Tested for the National Toxicology Program. Environmental and Molecular Mutagenesis (1994) 2351-63.

Gage J.C. 1970: The subacute inhalation toxicity of 109 industrial chemicals. Brit. J. industr. Med., 27:1-18.

Geyer H., Scheunert I. and Korte F. 1985: The effects of organic environmental chemicals on the growth of the alga Scenedesmus subcapitatus: a contribution to environmental biology. Chemosphere, 14(9):1355-1369.

Greim H., Bonse G., Radwan Z., Reichert D., Henschler D. 1975: Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. Biochemical Pharmacology, 24:2013-2017. Pergamon Press.

Heitmuller P.T., Hollister T.A. and Parrish P.R. 1981: Acute toxicity of 54 Industrial Chemicals to Sheepshead Minnows (*Cyprinodon variegatus*). Bull. Environm. Toxicol., 27:596-604.

Hong C.B., Winston J.M., Thornburg L.P. and Lee C.C. 1981: Follow-up study on the carcinogenicity on vinyl chloride and vinylidene chloride in rats and mice : tumor incidence and mortality subsequent to exposure. Journal of Toxicology and Environmental Health, 7:909-924.

IARC 2019: Some Chemicals That Cause Tumours of the Urinary Tract in Rodents. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 119

Jaeger R.J., Conolly R.B., and Murphy S.D. 1974: Effect of 18-hr fast and glutathione depletion on 1,1-dichloroethylene-induced hepatotoxicity and lethality in rats. Exp. mol. Pathol., 20: 187-198.

Jeffers P.M. et al. 1989: Homogeneous Hydrolysis Rate Constant for Selected Chlorinated Methanes, Ethanes, Ethenes, and Propanes. Environmental Science Technology (23):8, 965–969

Jenkins L.J., Trabulus Jr.M.J. and Murphy S.D. 1972: Biochemical effects of 1,1-Dichloroethylene in Rats: Comparison with Carbon Tetrachloride and 1,2-Dichloroethylene. Toxicology and applied pharmacology, 23:501-510.

Jones B.K., Hathway D.E. 1978a: Differences in metabolism of Vinylidene Chloride between mice and rats. Br. J. Cancer, 37:411-417.

Jones B.K. And Hathway D.E. 1978b: Tissue-Mediated Mutagenicity Of Vinylidene Chloride In Salmonella Typhimurium TA1535. Cancer Letters, 5. 1-6

Kennedy G.L., Graepel Jr.G. and Graepel G.Jay 1991: Acute toxicity in the rat following either oral or inhalation exposure. Toxicology Letters, 56:317-326.

Koch R., Schlegelmich R. and Wolf H.U. 1988: Genetic effects of chlorinated ethylenes in the yeast Saccharomyces cerevisiae. Mutation Research, 206:209-216.

Laumbach A.D., Lee S., Wong J. and Streips U.N. 1977: Studies on the mutagenicity of vinyl chloride metabolites and related chemicals. Prev Detect Cancer Proc Int Symp 3RD 1976, 1:155-170.

LeBlanc GA 1980: Acute Toxicity of Priority Pollutants to Water Flea (Daphnia magna). Environm. Contam. Toxicol., 24:684-691.

Lee C.C., Bhandari J.C., Winston J. M., House W. B., Peters P. J., Dixon R. L. and Woods J. S. 1977: Inhalation Toxicity of Vinyl Chloride and Vinylidene Chloride. Environmental Health Perspectives, 21:25-32.

Lee C.C., Bhandari J.C., Winston J. M., House W. B., Peters P. J., Dixon R. L. and Woods J. S. 1978: Carcinogenicity of Vinyl Chloride and Vinylidene Chloride, Journal of Toxicology and Environmental health, 4:15-30.

Lide, D.R. (ed.) 2000: CRC Handbook of Chemistry and Physics, 81st Ed., Boca Raton, FL, CRC Press, p. 16-43

Maltoni C., Cotti G., Morisi L. and Chieco P. 1977: Carcinogenicity bioassays of vinylidene chloride Research plan and early results. Medicina Del Lavoro, 68(4) 241-262.

Maltoni C., Cotti G., and Chieco P. 1984: Chronic toxicity and carcinogenicity bioassays of vinylidene chloride, Acta oncologica 5 (2).

Mc Gregor D., Brown A.G., Cattanach P., Edwards I., McBride D., Riach C., Shepherd W. and Caspary W.J. 1991: Responses of the L5178Y Mouse Lymphoma Forward Mutation Assay: V. Gases and Vapors. Environmental and Molecular Mutagenesis, 17:122-129.

Mortelmans K., Haworth S., Lawlor T., Speck W., Tainer B. and Zeiger E. 1986: Salmonella mutagenicity Tests: II. Results from the testing of 270 chemicals. Environmental Mutagenesis,8(sup.7):1-119.

National Toxicology Program 1982: Technical report No. 228, Carcinogenesis bioassay of vinylidene chloride (CAS NO. 75-35-4) in F344 rats and B6C3F1 mice (gavage study) (publication), U.S. Department of health and human services. Public Health Service. National Institute of Health.

National Toxicology Program 2015: Technical Report NTP TR 582; Toxicology and carcinogenesis of vinylidene chloride (CAS No. 75-35-4) in F344/N rats and B6C3F1/N mice (inhalation studies); National Institutes of Health, Public Health Service, U.S. Department on Health and Human Services.

Oesch, F., Protic-Sabljic, M., Friedberg, T., Klimisch, H.J., and Glatt, H.R. 1983: Vinylidene chloride: changes in drug metabolising enzyme, mutagenicity and relation to its targets for carcinogenesis. Carcinogenesis, 4: 1031-1038.

Howard P.H. (Ed.) 1989: Handbook of Environmental Fate and Exposure Data for Organic Chemicals, Volume I Large production and Priority Pollutants, Lewis Publishers, p. 561

Ponomarkov V., Tomatis L. 1980: Long-Term Testing of Vinylidene Chloride and Chloroprene for Carcinogenicity in Rats. Oncology, 1980, 37, 136-141.

Prendergast J.A., Jones R.A., Jenkins L.J. Jr. and Siegel J. 1967: Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. Toxicology and applied pharmacology, 10:270-289.

Quast J.F., Humiston C.G., Wade C.E., Ballard J., Beyer J.E., Schwetz R.W. and Norris J.M 1983: A chronic toxicity and oncogenicity study in rats and subchronic toxicity study in dogs on ingested vinylidene chloride. Fundamental and applied toxicology 1983, 3:55-62.

Quast J.F., McKenna M.J., Rampy L.W. and Norris J.M. 1986: Chronic toxicity and oncogenicity study on inhaled vinylidene chloride in rats. Fundamental and applied toxicology 1986, 6:105-144.

Rampy L. W., Quast J. F., Humiston C. G., Balmer M. F. and Schwetz B. A. 1977: Interim Results of Two-Year Toxicological Studies in Rats of Vinylidene Chloride Incorporated in the Drinking Water or Administered by Repeated Inhalation. Environmental Health Perspectives, 21:33-43.

Roberts S.M., Jordan K.E., Warren D.A., Britt J.K. and James R.C. 2002: Evaluation of the carcinogenicity of 1,1-dichloroethylene (vinylidene chloride). Regulatory Toxicology and Pharmacology, 35:44-55.

Reitz R.H., Watanabe P.G., McKenna M.J., Quast J.F., Gehring P.J. 1980: Effects of Vinylidene Chloride on DNA Synthesis and DNA Repair in the Rat and Mouse: A Comparative Study with Dimethylnitrosamine. Toxicology and Applied Pharmacology (1980), 52, 357-370.

Roldan-Arjona T., Garcia-Pedrajas M.D., Luque-Romero F.L., Hera C. and Pueyo C. 1991: An association between mutagenicity of the Ara test of Salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons. Mutagenesis, 6(3):199-205.

Sawada M., Sofuni T. and Ishidate Jr. M. 1987: Cytogenetic studies on 1,1-dichloroethylene and its two isomers in mammalian cells in vitro and in vivo. Mutation Research, 187:157-163.

Schmidt-Bleeck F., Haberland W., Klein A.W. et Caroli S. 1982 : Steps towards environmental hazard assessment of new chemicals (including a hazard ranking scheme based upon directive 79/831/EEC). Chemosphere 11(4): 383-415.

Short R.D., Winston J.M., Minor J.L., Hong C.B., Seifter J., & Lee, C.C. 1977a: Toxicity of vinylidene chloride in mice and rats and its alteration by various treatments. J. Toxicol. environ. Health, 3: 913-921.

Short R.D., Minor J.L., Winston J.M. and Lee C-C. 1977b: A dominant lethal study in male rats after repeated exposures to vinyl chloride or vinylidene chloride. Journal of Toxicology and Environmental Health, 3:965-968.

Siegel, J., Jones, R.A., Coon, R.A., & Lyon, J.P. 1971: Effects on experimental animals of acute, repeated and continuous inhalation exposures to dichloroacetylene mixtures. Toxicol. appl. Pharmacol., 18: 168-174.

Simmon V.F. and Tardiff R.G. 1977: The mutagenic activity of halogenated compounds found in chlorinated drinking water. Water chlorination, environmental impact and health effects, 2:416-431.

Tabak H. et al. 1981: Biodegradability studies with organic priority pollutant compounds. Journal of the Water Pollution Control Federation 53(10): 1503–1518

United States Environmental Protection Agency 1985: Health assessment document for vinylidene chloride; Final report EPA/600/8-83/031F

United States Environmental Protection Agency June 2002: Toxicological review of 1,1-dichloroethylene (CAS No. 75-35-4), EPA/635/R02/002.

Van Duuren B.L., Goldschmidt B.M., Loewengart G., Smith A.C., Melchionne S., Seidman I. and Roth D. 1979: Carcinogenicity of Halogenated Olefinic and Aliphatic Hydrocarbons in Mice. Journal of National Cancer Institute, 63(6).

Verschueren K. 1996: Handbook of Environmental Data on Organic Chemicals, 3rd Ed., John Wiley & Sons, Inc., p 687

Waskell Lucy 1978: A study of the mutagenicity of anesthetics and their metabolites. Mutation Research, 57:141-153.

Wilson B.H. et al. 1986: Biotransformation of Select Alkylbenzenes and Halogenated Aliphatic Hydrocabons in Methanogenic Aquifer Material: A Microcosm Study. Environmental Science & Technology 20(10): 997-1002

World Health Organization Geneva, 2003: Concise International Chemical Assessment Document 51, 1,1-dichloroethylene (Vinylidene chloride).

World Health Organization Geneva, 2009: Principles and methods for the risk assessment of chemicals in food. Environmental health criteria 240. ISBN: 9789241572408. Updates (2020) of section 4.5 of Chapter 4.

14 ANNEXES

Confidential Annex

Annex I for study summaries