CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name: 1,3-dichloropropene [1];
(Z)-1,3-dichloropropene [2];
(E)-1,3-dichloropropene [3]

EC Number: **[1] 208-826-5; [2] 233-195-8; [3] 431-460-4**

CAS Number: **[1] 542-75-6; [2] 10061-01-5; [3] 10061-02-6**

Index Number: **602-030-00-5**

Contact details for dossier submitter: Member State Competent Authority - PL

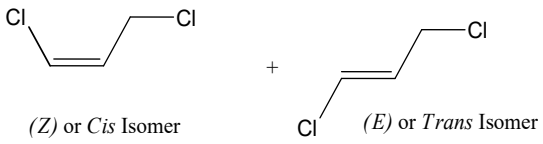
Version number: 5.1 **Date: March 2024**

CONTENTS

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(s) in the IUPAC nomenclature or other international chemical name(s)	IUPAC name: (*EZ*)-1,3-dichloropropene; CAS name: 1-Propene, 1,3-dichloro-
Other names (usual name, trade name, abbreviation)	1,3-D
ISO common name (if available and appropriate)	An ISO Common will not be allocated for this active substance
EC number (if available and appropriate)	208-826-5 [1] 233-195-8 [2] 431-460-4 [3]
EC name (if available and appropriate)	
CAS number (if available)	542-75-6 [1] 10061-01-5 [2] 10061-02-6 [3]
Other identity code (if available)	CIPAC number 675
Molecular formula	$C_3H_4Cl_2$
Structural formula	 *(Z)* or *Cis* Isomer *(E)* or *Trans* Isomer
SMILES notation (if available)	-
Molecular weight or molecular weight range	110.97 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not optically active but the geometric isomers are Cis 1,3-dichloroproprene Trans 1,3-dichloropropene
Description of the manufacturing process and identity of the source (for UVCB substances only)	*Not Applicable*
Degree of purity (%) (if relevant for the entry in Annex VI)	Minimum purity: 97 – 99% (cis + trans isomer total) Cis-1,3-dichloropropene: Minimum 40%

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] 1,3-dichloropropene (mixture of isomers), EC 208-826-5 CAS 542-75-6	97 – 99%	H226: Flammable liquid and vapour H301: Toxic if swallowed H311: Toxic in contact with skin H332: Harmful if inhaled H315: Causes skin irritation H319: Causes serious eye irritation H317: May cause an allergic skin reaction H335: May cause respiratory irritation H304: May be fatal if swallowed and enters airways H400: Very toxic to aquatic life H410: Very toxic to aquatic life with long lasting effects	H226: Flammable liquid and vapour H301: Toxic if swallowed H311: Toxic in contact with skin H331: toxic if inhaled H332: Harmful if inhaled H315: Causes skin irritation H319: Causes serious eye irritation H317: May cause an allergic skin reaction H335: May cause respiratory irritation H304: May be fatal if swallowed and enters airways H410: Very toxic to aquatic life with long lasting effects
[2] (Z)-1,3-dichloropropene (cis isomer) EC 233-195-8 CAS 10061-01-5		H226: Flammable liquid and vapour H301: Toxic if swallowed H311: Toxic in contact with skin H332: Harmful if inhaled H315: Causes skin irritation H319: Causes serious eye irritation H317: May cause an allergic skin reaction H335: May cause respiratory irritation H304: May be fatal if swallowed and enters airways H400: Very toxic to aquatic life H410: Very toxic to aquatic life with long lasting effects	Majority of notifiers: H226: Flammable liquid and vapour H301: Toxic if swallowed H311: Toxic in contact with skin H332: Harmful if inhaled H315: Causes skin irritation H319: Causes serious eye irritation H317: May cause an allergic skin reaction H335: May cause respiratory irritation H304: May be fatal if swallowed and enters airways H400: Very toxic to aquatic life H410: Very toxic to aquatic life with long lasting effects
[3] (E)-1,3-dichloropropene (trans isomer) EC 431-460-4 CAS 10061-02-6		Not current Annex VI entry. Proposed to be added to the group entry with current index number 602-030-00-5	H226: Flammable liquid and vapour H301: Toxic if swallowed H304: May be fatal if swallowed and enters airways H311: Toxic in contact with skin

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)
			H315: Causes skin irritation H317: May cause an allergic skin reaction H319: Causes serious eye irritation H331: Toxic if inhaled H335: May cause nasal cavity irritation H400: Very toxic to aquatic life H410: Very toxic to aquatic life with long lasting effects

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
No relevant impurities for the classification of the substance exist Note that some historical batches of 1,3-D were confounded by the use of the known genotoxic stabilizing agent epichlorohydrin, which has been replaced by epoxidized soybean oil (ESO) in the currently manufactured specification.				

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
None					

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
The test item is identified by the isomer(s) tested, namely (1,3-dichloropropene (mixture of isomers), (Z)-1,3-dichloropropene (cis isomer) or (E)-1,3-dichloropropene (trans isomer)) and the purity is documented in the individual sections where available.				

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: For substance with an existing entry in Annex VI of CLP

	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	602-030-00-5	1,3-dichloropropene; [1] (Z)-1,3-dichloropropene [2]	208-826-5 [1] 233-195-8 [2]	542-75-6 [1] 10061-01-5 [2]	Flam. Liq. 3 Acute Tox. 3 * Acute Tox. 3 * Acute Tox. 4 * Skin Irrit. 2 Eye Irrit. 2 Skin Sens. 1 Asp. Tox. 1 STOT SE 3 Aquatic Acute 1 Aquatic Chronic 1	H226 H301 H311 H332 H315 H319 H317 H304 H335 H400 H410	GHS02 GHS09 GHS08 GHS06 Dgr	H226 H301 H311 H315 H319 H317 H332 H304 H335 H410			Note D Note C
Dossier submitters proposal	602-030-00-5	1,3-dichloropropene; [1] (Z)-1,3-dichloropropene; [2] **Add** (E)-1,3-dichloropropene [3]	208-826-5 [1] 233-195-8 [2] **Add** 431-460-4 [3]	542-75-6 [1] 10061-01-5 [2] **Add** 10061-02-6 [3]	**Retain** Flam. Liq. 3 Acute Tox. 3 Acute Tox. 3 Skin Irrit. 2 Eye Irrit. 2 STOT SE 3 Asp. Tox. 1 **Modify** Skin Sens. 1A Acute Tox. 3 **Add** STOT SE 3 STOT RE 2 **Retain** Aquatic Acute 1 Aquatic Chronic 1	**Retain** H226 H301 H311 H315 H319 H335 H304 H317 **Modify** H331 **Add** H336 H373 (stomach) **Retain** H400 H410	**Retain** GHS02 GHS09 GHS08 GHS06 Dgr	**Retain** H226 H301 H311 H315 H319 H335 H304 H317 **Modify** H331 **Add** H336 H373 (stomach) **Retain** H410		**Add** oral: ATE = 85 mg/kg bw dermal: ATE = 330 mg/kg bw M=1 M=1	**Retain** Note D Note C

Resulting Annex VI entry if agreed by RAC and COM	602-030-00-5	1,3-dichloropropene; [1] (Z)-1,3-dichloropropene; [2] (E)-1,3-dichloropropene [3]	208-826-5 [1] 233-195-8 [2] 431-460-4 [3]	542-75-6 [1] 10061-01-5 [2] 10061-02-6 [3]	Flam. Liq. 3 Acute Tox. 3 Acute Tox. 3 Acute Tox. 3 Asp. Tox. 1 STOT SE 3 STOT RE 2 Skin Irrit. 2 Eye Irrit. 2 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H226 H331 H311 H301 H304 H335 H336 H373 (stomach) H315 H319 H317 H400 H410	GHS02 GHS09 GHS08 GHS06 Dgr	H226 H331 H311 H301 H304 H335 H336 H373 (stomach) H315 H319 H317 H410		dermal: ATE = 330 mg/kg bw oral: ATE = 85 mg/kg bw M=1 M=1	Note D Note C

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Harmonised classification proposed	Yes
Flammable solids	Hazard class not applicable	No
Self-reactive substances	Data lacking	Yes
Pyrophoric liquids	Data conclusive but not sufficient for classification	Yes
Pyrophoric solids	Hazard class not applicable	No
Self-heating substances	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Data conclusive but not sufficient for classification	Yes
Oxidising solids	Hazard class not applicable	No
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data lacking	Yes
Acute toxicity via oral route	Harmonized classification proposed	Yes
Acute toxicity via dermal route	Harmonized classification proposed	Yes
Acute toxicity via inhalation route	Harmonized classification proposed	Yes
Skin corrosion/irritation	Harmonized classification proposed	Yes
Serious eye damage/eye irritation	Harmonized classification proposed	Yes
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Harmonized classification proposed	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification.	Yes
Carcinogenicity	Data conclusive but not sufficient for classification.	Yes
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	Harmonized classification proposed	Yes
Specific target organ toxicity-repeated exposure	Harmonized classification proposed	Yes
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Harmonized classification proposed	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification.	Yes

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

1,3-dichloropropene has been evaluated by the technical committee C&L and the resulting classification according to Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures is reported above. The existing CLP Annex VI entry is a group entry for 1,3-dichloropropene and (Z)-1,3-dichloropropene.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

1,3-dichloropropene is proposed for approval as an active substance in the meaning of Regulation EC 1107/2009 therefore is subject to harmonised classification and labelling according to Article 36 CLP Regulation.

5 IDENTIFIED USES

The active substance is proposed for approval under Regulation (EC) No 1107/2009 as a nematicide on tomatoes and curcurbits. Details can be found in volume 1 of the European Draft Assessment Report. 1,3-dichloropropene (multi-constituent substance (E + Z isomers) has also industrial uses as an intermediate (no toxicological data in the REACH registration dossier). There are also REACH registration dossiers for E and Z isomers of 1,3-dichloropropene. The E and Z isomers of 1,3-dichloropropene are under the scope of this CLH proposal; the existing classification is proposed to be amended for the entire group entry covering the substance 1,3-dichloropropene, and E and Z isomers.

6 DATA SOURCES

European dossier in support of the approval under Regulation (EC) No 1107/2009, the relevant sections of which are reproduced in the present dossier.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	cis-isomer: slightly yellow clear liquid trans-isomer: clear colourless liquid at 20°C with odour of chlorinated solvent. Technical: clear colourless liquid with odour of chlorinated solvents.	Anonymous 1 (1990); Anonymous 2 (1998); Anonymous 3 (1998)	Observed
Melting/freezing point	cis-isomer: freezing point – 85 ºC (188 K) trans-isomer: < –25 ºC (lowest temperature achieved in the test).	Anonymous 1 (1990); Anonymous 2 (1998)	Measured EEC Method A.1
Boiling point	cis-isomer: 103.8 – 105.2 ºC trans-isomer: 114.5 ºC.	Anonymous 1 (1990); Anonymous 2 (1998)	Measured EEC Method A.2
Relative density	cis-isomer: Relative density (D234) = 1.221 trans-isomer: Relative density (D234) = 1.23	Anonymous 1 (1990); Anonymous 2 (1998)	Measured EEC Method A.3
Vapour pressure	cis-isomer: 3760 Pa at 20ºC 4850 Pa at 25ºC trans-isomer: 2982 Pa at 25ºC	Anonymous 1 (1990); Anonymous 2 (1998)	Measured EEC Method A.4
Surface tension	cis-isomer: 69.6 ± 0.4 mN/m at 20 °C (90% saturated solution) - not surface active trans-isomer: 61.0 mN/m at 21 °C (1 g/L solution) - not surface active	Anonymous 4 (2005); Anonymous 2 (1998)	Measured EEC Method A.5

Property	Value	Reference	Comment (e.g. measured or estimated)
Water solubility	cis-isomer (20 ºC): 2.45 g/L trans-isomer (20 ºC): 2.52 g/L	Anonymous 1 (1990); Anonymous 2 (1998)	Measured in purified water (tested substance is not an ionisable compound thus water solubility is not pH dependent) EEC Method A.6
Partition coefficient n-octanol/water	Log Kow cis-isomer: 1.82 at 20°C Log Kow trans-isomer: 2.1 at 20°C	Anonymous 1 (1990); Anonymous 2 (1998)	Measured EEC Method A.8
Flash point	cis-isomer: Flash point: 28.5 ºC. Technical: Flash point 27.0 ºC.	Anonymous 1 (1990); Anonymous 3 (1998)	Measured EEC Method A.9
Flammability	cis-isomer does not evolve highly flammable gases on contact with water. Cis-1,3-dichloropropene did not ignite during dropping or within five minutes of setting in any of the replicates tests. Cis-1,3-dichloropropene is non-pyrophoric. Technical: Non-flammable (contact with water)	Anonymous 1 (1990); Anonymous 3 (1998)	Measured EEC Method A.12
Explosive properties	cis-isomer does not have any explosive properties. Technical: Technical 1,3-dichloropropene is not explosive. DSC Scan of Telone 2 Technical mixed isomer (purity >95%) between 40 and 400°C shows no exotherms are present	Anonymous 1 (1990); Anonymous 3 (1998) Anonymous 7 (1991)	Measured EEC Method A.14
Self-ignition temperature	cis-isomer auto ignition temperature = 555 ± 5ºC. Technical: None below 400˚C	Anonymous 1 (1990); Anonymous 3 (1998)	Measured EEC Method A.15
Oxidising properties	Non oxidising	Anonymous 5 (2005)	Measured EEC Method A.21
Granulometry	n.a.	-	-
Stability in organic solvents and identity of relevant degradation products	Solvent g/L Methanol > 250 g/L Acetone > 250 g/L Xylene > 250 g/L 1,2-dichloroethane > 250 g/L Ethyl acetate > 250 g/L n-heptane> 250 g/L n-octanol > 250 g/L	Anonymous 3 (1998)	Measured at 25°C MT 181
Dissociation constant	Not applicable for a non-ionisable compound.	--	
Viscosity	*Kinematic Viscosity:* 0.636 mm^2s^{-1} at 20°C 0.544 mm^2s^{-1} at 40°C *Dynamic Viscosity:* Newtonian 0.769 mPa.s at 20°C 0.658 mPa.s at 40°C	Anonymous 6 (1997)	Measured OECD TG 114

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EC A14 Test item: Technical material (purity 98.7%) GLP	Technical 1,3-dichloropropene is not explosive	-	Anonymous 3 (1998) DAR, Spain, 2018
EC A14 Test item: cis-isomer (purity 98.1%)	The cis-isomer of 1,3-dichloropropene does not have any explosive properties	-	Anonymous 1 (1990) DAR, Spain, 2018
DSC Scan	DSC Scan of Telone 2 Technical mixed isomer (purity >95%) between 40 and 400°C shows no exotherms are present	This report was not required for the EU plant protection submission (Spain, 2018)	Anonymous 7 (1991)

8.1.1 Short summary and overall relevance of the information provided on explosive properties

The molecule 1,3-Dichloropropene does not contain chemical groups that are associated with explosive properties based on the examples of such groups given in Table A.6.1 in Appendix 6 of the UNRTDG, Manual of Tests and Criteria. In addition, the screening procedure (EC method A.14) was used to derive no thermal sensitivity (effect of flame, Koenen test), no mechanical sensitivity (shock, Fall Hammer test) and no mechanical sensitivity (friction, Friction test). The molecule 1,3-Dichloropropene is a low boiling point liquid (103 – 114°C) and a DSC of the mixed isomer material between 40 and 400°C show no exotherms throughout that temperature range. All of the evidence cited above indicate that the substance has no potential for explosive properties

8.1.2 Comparison with the CLP criteria

The molecule 1,3-Dichloropropene does not contain chemical groups that are associated with explosive properties based on the examples of such groups given in Table A.6.1 in Appendix 6 of the UNRTDG, Manual of Tests and Criteria. Accordingly the acceptance procedure for the hazard class ‘explosives’ need not be applied per Section 2.1.4.3 of the CLP regulation.

1,3-dichloropropene does not meet CLP criteria for classification as an explosive substance.

8.1.3 Conclusion on classification and labelling for explosive properties

1,3-dichloropropene is not classified for explosive properties according to the CLP criteria.

8.2 Flammable gases (including chemically unstable gases)

Not relevant, as 1,3-dichloropropene is a liquid.

8.3 Oxidising gases

Not relevant, as 1,3-dichloropropene is a liquid.

8.4 Gases under pressure

Not relevant, as 1,3-dichloropropene is a liquid.

8.5 Flammable liquids

Table 10: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
EC A9	Technical 1,3-dichloropropene: Flash point = 27.0ºC.	-	Anonymous 3 (1998) DAR, Spain, 2018
EC A9	Cis-isomer of 1,3-dichloropropene: Flash point: 28.5ºC.	-	Anonymous 1 (1990) DAR, Spain, 2018

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

According to test EC A9, the flash point of technical 1,3-dichloropropene is 27.0°C and the flash point of the cis-isomer of 1,3-dichloropropene is 28.5°C. The presented studies are relevant.

8.5.2 Comparison with the CLP criteria

According to Regulation (EC) No 1272/2008 (CLP), 1,3-dichloropropene is classified as flammable liquid Category 3, as it has a flash point ≥ 23°C and ≤ 60°C.

8.5.3 Conclusion on classification and labelling for flammable liquids

According to Regulation (EC) No 1272/2008 (CLP), 1,3-dichloropropene is classified as flammable liquid category 3 (H226: Flammable liquid and vapour).

8.6 Flammable solids

Not relevant, as 1,3-dichloropropene is a liquid.

8.7 Self-reactive substances

8.7.1 Comparison with the CLP criteria

According to criteria of CLP regulation the classification procedures for self-reactive substances and mixtures need not be applied if there are no chemical groups present in the molecule associated with explosive or self reactive properties. Examples of such groups are given in Tables A6.1 and A6.2 in Appendix 6 of the UN RTDG, Manual of Tests and Criteria. 1,3-dichloropropene does not contain any of above groups therefore the substance does not meet the CLP criteria for classification as self-reactive substance.

8.7.2 Conclusion on classification and labelling for self-reactive substances

1,3-dichloropropene is not classified as a self-reactive substance.

8.8 Pyrophoric liquids

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Not relevant, as no data provided.

8.8.2 Comparison with the CLP criteria

It is not necessary to consider 1,3-dichloropropene for classification in this hazard class, as experience in manufacture and handling demonstrates that the substance does not ignite spontaneously on contact with air at normal temperatures.

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

1,3-dichloropropene is not classified as a pyrophoric liquid.

8.9 Pyrophoric solids

Not relevant, as 1,3-dichloropropene is a liquid.

8.10 Self-heating substances

Table 11: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EC A15	Technical 1,3-Dichloropropene does not show any auto-ignition properties at temperatures up to 400ºC	-	Anonymous 3 (1998) DAR, Spain, 2018
EC A2	Boiling point cis-isomer: 103.8 – 105.2 ºC trans-isomer: 114.5 ºC.		Anonymous 1 (1990) DAR, Spain, 2018 Anonymous 2 (1998) DAR, Spain, 2018
DSC Scan	DSC Scan of Telone 2 Technical mixed isomer (purity >95%) between 40 and 400°C shows no exotherms are present	This report was not required for the EU plant protection submission, Spain 2018	Anonymous 7 (1991)

8.10.1 Short summary and overall relevance of the provided information on self-heating properties

The UN N4 test for a self-heating substance is defined as the ability of the substance to undergo oxidative self-heating as determined by exposure to air at temperatures of 100°C, 120°C, and 140°C. The molecule 1,3-Dichloropropene is a low boiling point liquid (103 – 114°C) and a DSC of the mixed isomer material between 40 and 400°C show no exotherms throughout that temperature range (Table 11). A study conducted in accordance with EC method A15 is available. In this study,1,3-dichloropropene technical did not self-ignite up to a maximum of 400°C. It is not possible to conduct the UN N4 test for self-heating with a liquid substance.

8.10.2 Comparison with the CLP criteria

According to the ECHA Guidance on the Application of the CLP Criteria (version 5.0 July 2017), the test method A.15 is not deemed appropriate to evaluate the self-heating property towards a CLP classification. However, substances with a low melting point (< 160°C) should not be considered for classification in this hazard class. 1,3-Dichloropropene is a liquid with a boiling point in the range 103 – 114°C.

In general, the phenomenon of self-heating applies only to solids, therefore liquids are not classified as self-heating.

8.10.3 Conclusion on classification and labelling for self-heating properties

1,3-Dichloropropene is not classified as a self-heating substance.

8.11 Substances which in contact with water emit flammable gases

Table 12: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
EC A12	Technical 1,3-dichloropropene (mixture of cis and trans) does not produce flammable gases on contact with water.	-	Anonymous 3 (1998) DAR, Spain, 2018
EC A12	The cis-isomer of 1,3-dichloropropene does not produce flammable gases on contact with water	-	Anonymous 1 (1990) DAR, Spain, 2018

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

According to test EC A12, neither technical 1,3-dichloropropene nor the cis-isomer of 1,3-dichloropropene emit flammable gases on contact with water. The presented studies are relevant.

8.11.2 Comparison with the CLP criteria

The classification procedure for this class need not be applied if the chemical structure of the substance does not contain metals or metalloids based on the chemical structure of the substance 1,3-dichloropropene the hazard class is not applicable.

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

1,3-dichloropropene is not classified as a substance which in contact with water emits flammable gases.

8.12 Oxidising liquids

Table 13: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
EC A21	Not oxidising		Anonymous 5 (2005) DAR, Spain, 2018

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

According to test EC A21, technical 1,3-dichloropropene is not an oxidising liquid. The presented studies are relevant.

8.12.2 Comparison with the CLP criteria

The hazard criteria of CLP regulation for oxidising liquids need not be applied to 1,3-dichloropropene based on an evaluation of its chemical structure. Although the substance contains chlorine, this element is bonded

only to carbon. The structure contains no other groups or elements associated with oxidising properties. Therefore the classification procedure for this class shall not apply according to CLP Annex I (section 2.13.4.1).

8.12.3 Conclusion on classification and labelling for oxidising liquids

1,3-dichloropropene is not classified as an oxidising liquid.

8.13 Oxidising solids

Not relevant, as 1,3-dichloropropene is a liquid.

8.14 Organic peroxides

Not relevant, as 1,3-dichloropropene is not an organic peroxide.

8.15 Corrosive to metals

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No data available

8.15.2 Comparison with the CLP criteria

A substance is classified as corrosive to metals using the test method outlined in section 37.4 of the UN RTDG Manual of Tests and Criteria. No data are available to indicate that 1,3-Dichloropropene is corrosive to metals. However, based on the experience in manufacture handling, packaging, and shelf-life storage the substance 1,3-Dichloropropene does not materially damage metallic (Stainless steel) containers.

No corrosion rate data is available for a comparison with the CLP criteria.

8.15.3 Conclusion on classification and labelling for corrosive to metals

No classification is proposed.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

While toxicokinetic data relevant to the assessment of mutagenicity and carcinogenicity are included in sections 10.8 and 10.9 of this report, other toxicokinetic data have been included in this section.

Table 14: Summary of animal studies on Toxicokinetics

Method	Results	Remarks	Reference
Study conducted prior to guideline. Six Carworth Farm E strain rats of each sex were housed in glass metabolism cages and cis-1,3-dichloro(2-^{14}) propene (2.51 mg, 7.98 µCi in 0.5 ml of arachis oil) was administered orally by gavage. The excretion of radioactivity in the urine, faeces and expired air was measured for four days and the radioactivity remaining in the gut, skin and carcass at four days was determined. Study conducted prior to GLP	cis-1,3-dichloro(2-^{14}) propene was rapidly metabolized by the rats and the metabolites were eliminated in the urine (83.1%), faeces (2.6%) and as carbon dioxide (3.8%). Only traces of radioactivity remained in the rats after four days	cis-1,3-dichloro(2-14) propene was synthesized with a specific gravity of 3.18 µCi/mg (0.35 mCi/mole). The metabolism of the compound is extremely rapid. The principal route of excretion of radioactivity was the urine (83.1%), almost all of which 80.7% of the administered dose occurred in the first 24 hours. Only trace amounts (0.5% in female rats and 1.4% in male rats) of radioactivity remained in the carcass after the four day period of the study. In comparison with the metabolism of 1.2-dichloropropane relatively small quantities of radioactivity were eliminated as carbon dioxide and as other volatile materials. The recoveries of radioactivity from two animals (male 1 and female 6) were poor but in view of the high vapour pressure of the compound and the consequent difficulty of accurate oral dosing the mean recovery of 91% is satisfactory. It is likely that 5~10% of the dose was lost as volatile materials soon after dosing probably as unchanged 1.3-dichloropropene.	Anonymous 70 (1969)
No guidelines followed Two female Wistar rats were each given a single oral dose of (Z or cis)-1,3-dichloro(2-14)propene (ca. 5mg, approximately 20 mg/kg bodyweight) in corn oil (0.7ml). Urine and faeces were collected separately. The metabolites in the urine were separated and estimated by chromatography and radioassay. The identity of the major metabolite was determined by physico-chemical techniques. Some in vitro metabolism work was also performed in order to identify the product of the enzyme-catalysed reaction between (Z or cis)-DCP and glutathione. GLP - No	The major urinary metabolite of (Z or cis)-DCP was identified as N-acetyl-S-[(Z)-3-chloroprop-2-enyl]cysteine (the mercapturic acid of (Z or cis)-DCP). (Z or cis)-DCP was also shown to react with glutathione in the presence of rat liver cytosol to produce S(Z)-3-chloroprop-2-enyl]glutathione.	The 0-24h urine was analysed directly by tlc in solvent systems a and b followed by radioscanning and autoradiography.This revealed three metabolites UI - U3 in the relative proportions: Ul - 92%, U2 – 3% and U3 - 5%. The polar metabolites U2 and U3 were present in small amounts relative to Ul and chromatographed poorly (broad peaks and low Rf values). U2 and U3_may not represent single radiocomponents since tlc in solvent c gave very broad bands which could be attributed to slight separation of radiocomponents or streaking on the tlc plate. The components did not appear to react with diazomethane or hot methanol/hydrogen chloride. Metabolite U1 was isolated as its methyester. The compound was analysed by mass spectroscopy. The presence of one chlorine atom in the molecule was revealed by the characteristic intensity ratios of the Mand M+2 peaks. A molecular ion of 251/253 suggested that the structure of the metabolite ester was methyl-N-acetyl-S-Z)-3-chloroprop-2-eny1)cysteine (IV).	Anonymous 71 (1978)

		The 360 MHz proton nmr spectrum similarly confirmed the identity of U1 as the mercapturic acid of (Z or cis)-DCP derivatised as the methylester. They were confirmed by spin decoupling experiments. In vitro studies - Tlc analysis of the in vitro incubations of rat liver cytosol glutathione and [^{14}Cl(Z)-DCP showed that a single polar radioactive compound was produced. In order to produce larger quantities of the material for structural elucidation by nmr analysis an incubation was performed on a larger scale. The conjugate was identified as S-((Z)-3-chloroprop-2-enyl)glutathione from interpretation. Spin decoupling experiments were performed in order to assign absolutely the resonance positions. This material and that obtained from the small scale incubation were shown to be identical by co-chromatography in solvent systems b, d and f	
Study – No guideline followed trans-1,3-Dichloro[2-^{14}C]propene was administered orally to twelve rats. The excretion of radioactivity in the urine, faeces and expired carbon dioxide was measured for four days. The rats were then sacrificed and the radioactivity remaining in the gut, skin and carcass determined. A second experiment to measure non-carbon dioxide radioactivity in the expired air was carried out using two rats. GLP - No	trans-l.3-Dichloro[2-^{14}C]propene was rapidly metabolized by the rat. The radioactivity was eliminated in the urine (58.0% of the administered dose over 4days) in the faeces (2.2%) and as carbon dioxide (23.5%). Only traces of radioactivity remained in the animal after four days.	trans-1,3-Dichloro[2-^{14}C]propene was synthesized with a specific activity of 3.15 µCi/ mg (0.35 mCi/mmole) and its purity was greater than 99% (confirmed by gas-liquid chromatography)	Anonymous 72 (1978)

In this study, six Carworth Farm E strain rats of each sex were housed in glass metabolism cages and cis-1,3-dichloro(2-^{14}) propene (2.51 mg, 7.98 µCi in 0.5 ml of arachis oil) was administered orally by gavage. The excretion of radioactivity in the urine, faeces and expired air was measured for four days and the radioactivity remaining in the gut, skin and carcass at four days was determined.

The metabolism of the compound is extremely rapid. The principal route of excretion of radioactivity was the urine (83.1%), almost all of which 80.7% of the administered dose occurred in the first 24 hours. Only trace amounts (0.5% in female rats and 1.4% in male rats) of radioactivity remained in the carcass after the four day period of the study. In comparison with the metabolism of 1.2-dichloropropane (Moss.1969) relatively small quantities of radioactivity were eliminated as carbon dioxide and as other volatile materials. The recoveries of radioactivity from two animals (male 1 and female 6) were poor but in view of the high vapour pressure of the compound and the consequent difficulty of accurate oral dosing the mean recovery of 91% is satisfactory. It is likely that 5~10% of the dose was lost as volatile materials soon after dosing probably as unchanged 1.3-dichloropropene.

To summarize, cis-1,3-dichloro(2-^{14}) propene was rapidly metabolized by the rats and the metabolites were eliminated in the urine (83.1%), faeces (2.6%) and as carbon dioxide (3.8%). Only traces of radioactivity remained in the rats after four days.

The second study was conducted to characterise and estimate the urinary metabolites of (Z or cis)-1,3-dichloropropene in urine following a single oral dose. Two female Wistar rats were each given a single oral dose of (Z or cis)-1,3-dichloro(2-14)propene (ca. 5mg, approximately 20 mg/kg bodyweight) in corn oil (0.7ml). Urine and faeces were collected separately. The metabolites in the urine were separated and estimated by chromatography and radioassay. The identity of the major metabolite was determined by physico-chemical techniques. Some in vitro metabolism work was also performed in order to identify the product of the enzyme-catalysed reaction between (Z or cis)-DCP and glutathione. The major urinary metabolite of (Z or cis)-DCP was identified as N-acetyl-S-[(Z)-3-chloroprop-2-enyl]cysteine (the mercapturic acid of (Z or cis)-DCP). (Z or cis)-DCP was also shown to react with glutathione in the presence of rat liver cytosol to produce S(Z)-3-chloroprop-2-enyl]glutathione.

In the second study, six rats of each sex were housed in glass metabolism cages. and trans-1,3-Dichloro[2-^{14}C]propene (2.7 mg, 8.5 µCi in 0.5 ml. of arachis oil) was administered to each animal by stomach tube. Expired air from three animals of each sex was drawn through two absorbers each containing 500 ml of 5N sodium hydroxide solution. The absorbed carbon dioxide in the first bottle was released by acidification of 1 ml of solution and trapped and radioassayed in scintillator solution. Solution from the second bottle (1 ml) was added directly to scintillator for radioassay. Daily excretion of radioactivity in the urine over four days was measured by making up each urine to a standard volume and transferring 1ml of solution to scintillator for radioassay. Samples of homogenized faeces were combusted in a furnace and the carbon dioxide absorbed in scintillator for assay of radioactivity. After four days the animals were killed and radioactivity in skin, gut and remaining carcass measured as for faeces. Results were calculated accordingly. A second experiment, to measure non-carbon dioxide radioactivity in the expired air was carried out using two female rats. Each rat was dosed with trans-1,3-dichloro[2-^{14}C]propene (2.3 mg, 7.3 µCi in 0.5 ml of arachis oil) by stomach tube. The animals were housed in metabolism cages as in the previous experiment. The expired air was first drawn through two absorbers each containing 500 ml of toluene cooled to -20°C before being passed through two traps containing 5N sodium hydroxide. The animals were killed after 24 hours. Duplicate 0.1 ml samples were taken from each carbon dioxide trap, blended with scintillator and assayed for radioactivity. Duplicate 1ml samples were taken from each toluene trap mixed with scintillator and assayed for radioactivity. The radioactivity in the urine was measured as described above. All radioassays were carried out by liquid scintillation counting using a Packard Tricarb Liquid Scintillation Spectrometer, Model3003.

trans-l.3-Dichloro[2-^{14}C]propene was rapidly metabolized by the rat. The radioactivity was eliminated in the urine (58.0% of the administered dose over 4days) in the faeces (2.2%) and as carbon dioxide (23.5%). Only traces of radioactivity remained in the animal after four days.

Table 15: Excretion of ^{14}C (% of administered dose)

Compound	Urine (%)	Faeces (%)	Retained ^{14}C (%)	CO_2 (%)	Volatile radioactivity trapped in toluene (%)
trans-l.3-Dichloro[2-^{14}C]propene	58.0	2.2	1.7	23.5	2.0 – 5.0

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 16: 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
OECD 401, no deviations GLP	Rat SD. Four groups of 5 Sprague-Dawley CFY rats by sex	The test material was Telone II 86/3293 (lot 0713054/062), with purity 97.2% of 1,3-Dichloropropene 200 mL. The solvent was polyethylene glycol 400 No information on isomer composition is available in the study report, however cis- 1,3-dichloropropene content≥45% is specified for Telone II	All the test animals were treated with a single oral dose of test material at doses of 75, 110, 170 and 250 mg/kg bw	**All animals LD_{50}:150 mg/kg** (110-170 mg/kg) Male LD_{50}: 130 mg/kg (110-170 mg/kg) Female LD_{50}: between 170 and 250 mg/kg **Dose mg/Kg** / **Number of deaths** (Male / Female) 75: 0/5 / 0/5 110: 0/5 / 0/5 170: 5/5 / 2/5 250: 5/5 / 5/5	Anonymous 8 (1986) DAR, Spain, 2018
No guidelines followed, study conducted according to the /Principles of GLP	Sprague-Dawley CD SD rats (males only)	Telone II (Lot/Refer. No. UB1716284A, TSN105628). Sample containing 51.4% cis- (Z-) and 45.8% trans- (E-) isomers for a total of 97.2% active ingredient by gas chromatograph	Groups of test animals were dosed in a split dosing regimen at doses (total) of 150, 225, 300 and 600 mg/kg using PEG 400 as a vehicle and another group dosed in a similar manner with rodent chow slurry.	Under the conditions of this study, the split dosing regimen reduced the acute oral toxicity of TELONE II. The LD_{50} of TELONE II in PEG 400 after oral gavage administration over four hour intervals was greater than 300 mg/kg bw. Using the same split dosing regimen, the LD_{50} of TELONE II in rodent chow slurry was estimated to be 424.3 mg/kg bw. This compares with an established acute **oral LD_{50} value of 130 mg/kg bw** for TELONE II in PEG 400 in male Sprague-Dawley rats	Anonymous 9 (2006) DAR, Spain, 2018

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 401 – dietary route of exposure - GLP	Sprague-Dawley Crl:CD(SD) rats. Groups of five male rats were provided with test diets formulated to supply 0, 300, 600, or 900 mg (1,3-D) per kg bw during a single 2-hour feeding period. The rats were given control feed for the remainder of the 14-day observation period.	Telone II microcapsules (Lot/Refer. No. M011608, TSN030336-0001). The purity of the test material was determined to have a 1,3 D loading of 29.1 ± 1.0(s)% by gas chromatography No information on isomer composition is available in the study report, however cis- 1,3-dichloropropene content≥45% is specified for Telone II	Groups of five male rats were provided with test diets formulated to supply 0, 300, 600, or 900 mg (1,3-D) per kg bw during a single 2-hour feeding period. Test material intake was only 47.3, 55.8, and 47.3 mg/kg bw for the targeted 300, 600, and 900 mg 1,3-D/kg bw groups, respectively	Under the conditions of this study, the maximum dietary dose of 55.8 mg 1,3-D/kg bw had no effect on the survival of male Crl:CD(SD) rats following a single 2-hour dietary exposure period.	Anonymous 141 (2008); DAR, Spain, 2018
OECD 401 – oral gavage No deviations GLP	4 groups of ten fasted Sprague Dawley rats (5 males and five females)	Cis 1,3-dichloropropene (Batch No. 87/RM/716) Purity not available	75, 110, 170 and 250 mg/kg body weight administered as a solution in polyethylene glycol 400 – PEG 400	**LD_{50} – All animals – 121** (107 – 137) mg/kg body weight. Males – 126 (108 – 148) mg/kg body weight Females – 117 (96 – 142) mg/kg body weight **Dose mg/Kg** / **Number of deaths** (Male / Female) 75: 0/5 / 0/5 110: 1/5 / 2/5 170: 5/5 / 5/5 250: 5/5 / 5/5	Anonymous 17 (1988)
EEC Method B1 No deviations GLP	Groups of five male and five female Fischer F344 rats were fasted overnight, weighed and given a single dose of the undiluted test material at doses of 75, 90, 120 and 160 mg/kg, by gavage, using a ball pointed cannula and syringe	Trans-1,3-Dichloropropene Batch No. TR88001 Purity 96.7%	75, 90, 120 and 160 mg/kg administered undiluted	The acute oral **LD_{50}** of the undiluted test material in rats was **94 mg/kg** [95% confidence interval 82 to 108 mg/kg].	Anonymous 18 (1988)

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance,</th><th>Dose levels, duration of exposure</th><th>Value
LD_{50}</th><th>Reference</th></tr>
<tr><td>OECD 401

No deviations

GLP</td><td>Groups of five male and five female Fischer F344 rats were fasted overnight, weighed and given a single dose of the undiluted test material (cis-1,3-dichloropropene) at doses of 38, 68, 90, 120, 160, and 213 mg/kg, by gavage</td><td>Cis-1-3-Dichloropropene

Batch No. ST88/253

Purity 96.9%</td><td>38, 68, 90, 120, 160, and 213 mg/kg administered undiluted</td><td>The acute oral LD_{50} of the undiluted test material in male rats was 93 mg/kg (95% C.I. 76 to 113 mg/kg), female rats was 78 mg/kg (95% C.I. 68 to 90 mg/kg) and combined sexes was 85 mg/kg (95% C.I. 76 to 96 mg/kg)
<table><tr><th rowspan="2">Dose mg/Kg</th><th colspan="2">Number of deaths</th></tr><tr><th>Male</th><th>Female</th></tr><tr><td>38</td><td>0/5</td><td>0/5</td></tr><tr><td>51</td><td>0/5</td><td>0/5</td></tr><tr><td>68</td><td>0/5</td><td>0/5</td></tr><tr><td>90</td><td>3/5</td><td>5/5</td></tr><tr><td>120</td><td>5/5</td><td>5/5</td></tr><tr><td>160</td><td>4/5</td><td>5/5</td></tr><tr><td>231</td><td>5/5</td><td>5/5</td></tr></table></td><td>Anonymous 23 (1989)</td></tr>
</table>

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

Acute toxicity of 1,3-Dichloropropene (1,3-D) was investigated after oral administration in rats. In the Anonymous 8 (1986) study (OECD Test Guideline 401, GLP) deaths were observed from 170 mg/kg bw in both sexes. The majority of deaths were noted 4-6 hours after dosing or on day one. Animals in all dose groups showed hunched posture and pilo-erection one and four hours after dosing. Decreased respiratory rate was also noted in the males treated with 75 mg/kg bw one hour after dosing and all surviving animals treated with 110 mg/kg bw and above showed decreased respiratory rate and lethargy at the one and four-hour observations. Occasional signs of ptosis and an isolated sign of diarrhoea were noted on the day of dosing in animals treated with 110 mg/kg bw, whilst ataxia, ptosis and increased salivation were noted in all survivors from the 170 and 250 mg/kg bw dose groups during this period. Increased lacrimation was apparent in two females treated with 250 mg/kg bw one hour after treatment, and one of these also showed diuresis at this time.

Animals treated with 75 mg/kg bw recovered and appeared normal on day one but those treated with 110 mg/kg bw continued to show hunched posture and pilo-erection at this time. Animals in the 110 mg/kg bw dose group appeared normal on day two and no further signs of toxicity were noted in either of these groups throughout the remainder of the study period. All males treated with 170 mg/kg bw had died by the final death check on the day of dosing but the females in this group showed prolonged signs of toxicity, including hunched posture, lethargy, pilo-erection, decreased respiratory rate, ptosis, diarrhoea, diuresis, ataxia, tiptoe gait, red/brown staining around the snout, occasional body tremors, emaciation and pallor of the extremities. One surviving female in this group recovered and appeared normal on day seven and throughout the remainder of the study period but the remaining two survivors had not fully recovered until day twelve. All animals treated with 250 mg/kg bw were found dead on the day of dosing or on day one.

Table 17 – Clinical Observations and Mortality Data

Dose Level (mg/kg)	Clinical observations	Number showing effects during day of dosing (hour)		Number showing effects during day of observation									Total mortality
		1	4	1	2	3	4	5	6	7	8	9-14	
75	Hunched posture	10	10	0	0	0	0	0	0	0	0	0	0/10
	Pilo-erection	10	10	0	0	0	0	0	0	0	0	0	
	Decreased respiratory rate	5	0	0	0	0	0	0	0	0	0	0	
	Normal	0	0	10	10	10	10	10	10	10	10	10	
	Dead	0	0	0	0	0	0	0	0	0	0	0	
110	Hunched posture	10	10	10	0	0	0	0	0	0	0	0	0/10
	Pilo-erection	10	10	10	0	0	0	0	0	0	0	0	
	Lethargy	10	10	0	0	0	0	0	0	0	0	0	
	Decreased respiratory rate	10	10	0	0	0	0	0	0	0	0	0	
	Diarrhoea	1	0	0	0	0	0	0	0	0	0	0	
	Ptosis	0	2	0	0	0	0	0	0	0	0	0	
	Normal	0	0	0	10	10	10	10	10	10	10	10	
	Dead	0	0	0	0	0	0	0	0	0	0	0	

Dose Level (mg/kg)	Clinical observations	Number showing effects during day of dosing (hour)		Number showing effects during day of observation									Total mortality
		1	4	1	2	3	4	5	6	7	8	9-14	
170	Hunched posture	10	9	5	5	5	5	4	4	3	3	2 < (days 9-11 only)	7/10
	Pilo-erection	10	9	5	5	5	5	4	4	3	3	2 < (days 9-11 only)	
	Lethargy	10	9	5	0	5	5	4	1	2	1	0	
	Decreased respiratory rate	10	9	5	5	5	1	0	1	2	1	0	
	Increased salivation	10	9	0	0	0	0	0	0	0	0	0	
	Ptosis	1	9	5	0	0	0	0	0	0	0	0	
	Ataxia	0	9	1	0	1	1	0	0	0	0	0	
	Diarrhoea	0	0	5	5	0	0	0	0	0	0	0	
	Diuresis	0	0	5	3	0	0	0	0	0	0	0	
	Tiptoe gait	0	0	5	0		2	0	0	0	0	0	
	Red/brown staining around snout	0	0	0	0		1	0	0	0	0	0	
	Occasional body tremors	0	0	0	0		1	0	0	0	0	0	
	Emaciation	0	0	0	0		1	0	0	0	0	0	
	Pallor of the extremities	0	0	0	0		0	0	1	0	0	0	
	Normal	0	0	0	0		0	0	0	1	1	1*	
	Dead	0	5+	0	0		0	1	0	0	0	1 (day9)	

+ = 4 animals found dead at afternoon death check approximately 6 hours after dosing
* = all animals normal on day 12

Dose Level (mg/kg)	Clinical observations	Number showing effects during day of dosing (hour)		Number showing effects during day of observation									Total mortality
		1	4	1	2	3	4	5	6	7	8	9-14	
250	Hunched posture	10	7	-	-	-	-	-	-	-	-	-	10/10
	Pilo-erection	10	7	-	-	-	-	-	-	-	-	-	
	Lethargy	10	7	-	-	-	-	-	-	-	-	-	
	Decreased respiratory rate	10	7	-	-	-	-	-	-	-	-	-	
	Increased salivation	10	7	-	-	-	-	-	-	-	-	-	
	Ptosis	10	7	-	-	-	-	-	-	-	-	-	
	Ataxia	0	7	-	-	-	-	-	-	-	-	-	
	Increased lacrimation	2	0	-	-	-	-	-	-	-	-	-	
	Diuresis	1	0	-	-	-	-	-	-	-	-	-	
	Normal	0	0	-	-	-	-	-	-	-	-	-	
	Dead	0	6*	4	-	-	-	-	-	-	-	-	

* = 3 animals found dead at afternoon check approximately 6 hours after dosing
- = all animals dead

The acute oral median lethal dose (LD_{50}) was found between 110 and 170, 170 and 250 mg/kg bw for male and female rats, respectively.

The second study (Anonymous 9, 2006) is not considered appropriate for classification purposes due to use of the split dosing regimen, while the acute oral toxicity is to be established on the basis of OECD guideline studies on single dose gavage test substance.

Test animals: Sprague-Dawley CD SD rats. Previous toxicity data indicated CD male rats were more sensitive than females to acute exposures to TELONE II. Therefore, only male rats were tested in this study. Test substance is administered by gavage. The dose regime is summarized in table below:

Table 18: Design of acute oral toxicity in rats

Phase	Group	Number of animals	Total dose (mg/kg)	Dose Conc. (mg/ml)	Total Dose Volume (ml/kg bw)	Dose level/Interval (mg/kg)	Dose Intervals	Vehicle
1	1	1	0	0	30	0	0, 1 and 2 hour	PEG 400
	2	3	150	5		50		
2	3	4	0	0	10	0	0, 4 and 8 hour	PEG 400
	4	3	150	15		50		
	5	3	225	22.5		75		
	6	3	300	30.03		100		
3	7	6	0	0	10	0	0, 4 and 8 hour	Rodent chow slurry
	8	3	300	30.03		100		
	9	3	600	60.06		200		

Control animals were dosed only with vehicle. Parameters evaluated during the 14-day observation period included body weights and clinical observations. A gross necropsy was conducted on all animals.

FINDINGS

Phase 1: All three animals receiving 150 mg/kg bw/day TELONE II/kg bw in PEG 400 at 0, 1 and 2 hours died by test day 2. Due to the mortality of these animals, a single PEG 400 vehicle control animal was dosed with the same volume (30 mg/ml) to determine if the relatively high dose volume contributed to the death of the TELONE II treated animals. The clinical signs in this control animal justified early termination of the

animal on day 1, indicating that the vehicle and/or dose interval significantly contributed to the toxicity in these animals.
***Phase 2**:* Clinical observations in the treated animals consisted of various combinations of decreased activity, soft faeces, perineal soiling, perioral soiling, and/or eyelids partially closed. Two of the PEG 400 vehicle control animals also had clinical signs on day 1 that included perineal soiling and/or soft/watery faeces. Clinical signs in all animals had resolved by test day 3. All animals had gained weight by the end of the study. Gross pathological findings consisted of an ulcer or a thickened area in the nonglandular mucosa in the stomach in two animals at the 150 mg/kg bw/day dose level. All animals dosed with 300 mg/kg bw/day had gross pathological findings that included thickening and/or ulcers in the nonglandular mucosa of the stomach, and/or adhesions of the nonglandular serosa of the stomach to the spleen. There were no gross pathological findings in the 0 or 225 mg/kg bw/day group. All animals survived and gained weight by the end of 14-day observation period.
***Phase 3**:* Clinical signs in these animals consisted of soft faeces, perineal soiling, perioral soiling, and/or decreased activity that resolved by test day 3. These clinical signs were accompanied by reduced body weight gain on test day 2 compared to controls. Body weight gain was similar to controls by the end of the study. There were no gross pathological findings in the 300 mg/kg bw/day group. All animals dosed with 600 mg/kg bw/day in rodent chow slurry died by test day 2. Prior to death, clinical signs noted on day 1 included decreased activity, perioral soiling, perineal soiling, soft faeces, and/or periocular soiling. Gross pathological findings in the 600 mg/kg bw/day animals included watery contents in the GI tract, fluid in the thoracic cavity, facial and/or perineal soiling, congestion and dilatation of the stomach.

Mortality data are summarised in the table below.

Table 19: Summary of mortality data

Total Dose (mg/kg)	Vehicle	Dose Interval	No. animals dosed	No. animals dead	Approximate Observed Time of Death (Day)
0	PEG 400	0, 1 and 2 hour	1	1	1
150	PEG 400	0, 1 and 2 hour	3	3	2
0	PEG 400	0, 4 and 8 hour	4	0	-
150	PEG 400	0, 4 and 8 hour	3	0	-
225	PEG 400	0, 4 and 8 hour	3	0	-
300	PEG 400	0, 4 and 8 hour	3	0	-
0	slurry	0, 4 and 8 hour	6	0	-
300	slurry	0, 4 and 8 hour	3	0	-
600	slurry	0, 4 and 8 hour	3	3	2

Under the conditions of this study, the split dosing regimen reduced the acute oral toxicity of TELONE II. The LD_{50} of TELONE II in PEG 400 after oral gavage administration over four hour intervals was greater than 300 mg/kg bw. Using the same split dosing regimen, the LD_{50} of TELONE II in rodent chow slurry was estimated to be 424.3 mg/kg bw.

In the third study (Anonymous 141, 2008), groups of five male rats were provided with test diets formulated to supply 0, 300, 600, or 900 mg (1,3-D) per kg bw during a single 2-hour feeding period. The rats were given control feed for the remainder of the 14-day observation period. Study parameters evaluated included daily cage-side observations, detailed clinical observations, body weights, feed consumption, and gross pathologic examinations.

All animals survived and appeared normal throughout the study and there were no gross pathological observations at test termination. Feed consumption on the day of exposure to treated food was significantly reduced by 86.5%, 91.7% and 95.3% in the 300, 600, and 900 mg 1,3-D/kg dose groups, respectively when compared to controls. As a result, test material intake was only 47.3, 55.8, and 47.3 mg/kg bw for the targeted 300, 600, and 900 mg 1,3-D/kg bw groups, respectively. All treated groups lost body weight by the following day, with a decrease of 6.1%, 7.6%, and 7.1% relative to the pre-exposure weight, for the 300, 600, and 900 mg 1,3-D/kg dose groups, respectively. The control animals, on the other hand, showed an increase in mean

body weight of 4.8% over the same period. When the animals were returned to untreated diet, all gained weight by test day 8 and 15 and feed consumption was comparable to controls for the remainder of the 14-day study.

Under the conditions of this study, the maximum dietary dose of 55.8 mg/kg bw had no effect on the survival of male rats following a single 2-hour dietary exposure. The maximum dietary dose achieved was lower than the targeted doses due to decreased feed consumption in all treated animals. Due to a lack of any observed toxicological response (behavioral or clinical signs) during or following exposure to the treated diets, the reduced feed consumption is probably due to palatability as opposed to systemic toxicity.

In the fourth study (Anonymous 17, 1988), the cis-isomer of 1,3-dichloropropene was tested for acute oral toxicity in groups of 10 rats (5 male and 5 female). Doses from 75 to 250 mg/kg were administered by oral gavage in a constant dose volume of polyethylene glycol 400. All rats showed non-specific signs of intoxication but survivors were normal 4 days after dosing. Animals dying during the study showed abnormalities of lung, liver and gastro-intestinal tract. Survivors of the 14 day observation period still showed evidence of damage to the gastro-intestinal tract at necropsy. The LD_{50} of this material was 126 mg/kg for males, 117 mg/kg for females and 121 mg/kg for the sexes combined (107-137 mg/kg 95% confidence limits).

In the fifth study (Anonymous 18, 1988), groups of five male and five female Fischer F344 rats were fasted overnight, weighed and given a single dose of the undiluted test material at doses of 75, 90, 120 and 160 mg/kg, by gavage, using a ball pointed cannula and syringe. Approximately three hours after dosing on Day l the animals were allowed food again ad libitum. A careful clinical examination was made three times daily for the first three days and once daily thereafter for the remainder of the 14- day observation period. The initial (Day l), Day 7 and Day 14 bodyweights were recorded, and changes in bodyweight calculated. All animals were subject to necropsy. Animals sacrificed for humane reasons during the study or surviving to the end of the study were killed by an intraperitoneal injection of sodium pentobarbitone. The cranial, thoracic and abdominal cavities and viscera were examined and any gross pathological changes recorded. The 14-day LD_{50}, 95% confidence interval and the dose-mortality slope were calculated using a method based on probit analysis (Finney, 1977).

The principal signs of reaction to treatment observed within 5 hours of oral administration of trans-1,3-dichloropropene were diarrhoea or voiding of soft faeces, cyanosis, increased lachrymation, a hunched posture, lethargy, piloerection and ante-mortem prostration. Other clinical signs included abasia, increased salivation and ataxia among rats dosed at 90 mg/kg and above and an isolated case of ante-mortem hypothermia. Piloerection, a hunched posture and an unkempt appearance were commonly apparent on Day 2 and Day 3 among rats treated at the low or intermediate dose-levels. The recovery of rats surviving the toxic effects of trans-1,3-dichloropropene was generally advanced by Day 4 although the unkempt appearance of many rats persisted for up to nine days after treatment. Bodyweight losses between Days 1 and 7 were recorded for one or two rats at all dose-levels. All surviving rats had gained weight relative to their Day 1 bodyweights by the end of the 14-day observation period.

The acute oral LD_{50} of the undiluted test material in rats was 94 mg/kg [95% confidence interval 82 to 108 mg/kg].

In this last OECD Test Guideline 401 (GLP) study (Anonymous 23, 1989), groups of five male and five female Fischer F344 rats were fasted overnight, weighed and given a single dose of the undiluted test material (cis-1,3-dichloropropene) at doses of 38, 68, 90, 120, 160, and 213 mg/kg, by gavage using a ball-pointed cannula and syringe. Approximately three hours after dosing on Day 1 the animals were allowed food again ad libitum. A careful clinical examination was made up to three times daily for the first three days and once daily thereafter for the remainder of the 14-day observation period. The initial Day 1, Day 7 and Day 14 bodyweights were recorded and changes in bodyweight calculated. All animals were subject to necropsy. Animals surviving to the end of the study were killed by an intraperitoneal injection of sodium pentobarbitone. The cranial, thoracic and abdominal cavities and viscera were examined, and any gross pathological changes recorded. The 14-day LD_{50}, 95% confidence interval and the dose-mortality slope were calculated using a method based on probit analysis (Finney, 1977).

Mortalities occurred on days 1, 2 and 3. Common signs of reaction to treatment observed among rats dosed at 38, 51 or 68 mg/kg were limited to voiding of soft faeces or diarrhoea within 4 hours of dosing and piloerection and/or an unkempt appearance during Day 2. The recovery of rats surviving oral administration of cis-1,3-dichloropropene was with a single exception (Rat 196 M), complete by Day 3. Only three rats survived treatment at 90 mg/kg and above. The majority of deaths occurred within four hours of dosing. Principal clinical signs observed prior to death were increased lachrymation and salivation, abasia or ataxia and diarrhoea. Less commonly lethargy, cyanosis and unkempt appearance were observed and there were isolated cases of hypothermia, piloerection and hunched posture. All surviving rats had gained weight relative to their Day 1 bodyweights by the end of the 14-day observation period. The principal macroscopic abnormalities found in decedents were abnormal contents, inflammation and haemorrhage of the stomach, darkening of the liver, lung congestion and discolouration of the renal medulla. No macroscopic abnormalities were found in rats that survived treatment.

The acute oral LD_{50} of the undiluted test material in male rats was 93 mg/kg (95% C.I. 76 to 113 mg/kg), female rats was 78 mg/kg (95% C.I. 68 to 90 mg/kg) and combined sexes was 85 mg/kg (95% C.I. 76 to 96 mg/kg).

Common clinical symptoms were body weight loss, lethargy, diarrhoea, lacrimation, palpebral closure, laboured respiration, facial soiling, respiratory difficulty, skin irritation and/or rough hair coat. The treatment related gross changes were stomach lesions and lung congestion.

10.1.2 Comparison with the CLP criteria

In acute oral toxicity studies conducted according to OECD TG 401 (or comparable methods i.e.: EEC Method B1), the LD_{50} values in rats were determined to be 150, 85-121 and 94 mg/kg bw for 1,3-D (mix of isomers), (Z)-1,3-D (cis-isomer) and (E)-1,3-D (trans-isomer) respectively (Anonymous 8 (1986), Anonymous 9 (2006), Anonymous 17 (1988), Anonymous 23 (1989) and Anonymous 18 (1988)).

According to the criteria of Regulation (EC) No. 1272/2008 (the CLP Regulation): substances for which the acute oral LD_{50} is > 50 mg/kg and ≤ 300 mg/kg bw require classification in Acute Toxicity Category 3 (H301) *"Toxic if swallowed."*

The evaluation of available experimental data on 1,3-D (mix of isomers), (Z)-1,3-D (cis-isomer - Anonymous 23, 1989) and (E)-1,3-D (trans-isomer - Anonymous 18, 1988) indicates that these substances have a comparable profile with respect to the potential for acute oral toxicity. These substances respectively meet the CLP criteria for classification in Acute Toxicity Category 3 (H301) "*Toxic if swallowed."*

Specific Concentration Limit of 85 mg/kg bw is proposed as the lowest acute oral LD_{50} of the undiluted test material in male and female rats based on valid, well-performed study by Anonymous 23, 1989.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on data presented, 1,3-D (mix of isomers), (Z)-1,3-D (cis-isomer) and (E)-1,3-D (trans-isomer) should be classified in accordance with the CLP Regulation as:

Acute oral toxicity Category 3, H301, Toxic if swallowed with ATE value = 85 mg/kg bw.

10.2 Acute toxicity - dermal route

Table 20: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
OECD 402, no deviations GLP	Four groups of ten animals, 5 male and 5 female Sprague-Dawley CFY strain rats	Telone II 86/3293 soil fumigant containing 97.2% 1,3-Dichloropropene; Lot 0713054/062. No information on isomer composition is available in the study report, however cis- 1,3-dichloropropene content ≥45% is specified for Telone II	All rats were treated with a single dermal dose of test material (occluded application) at doses of 500, 800, 1300 or 2000 mg/kg bw	The acute dermal **LD_{50} was calculated for all animals as 1200 mg/kg bw,** separately for males between 800 and 1300 mg/kg bw and for females between 1300 and 2000 mg/kg bw. **Dose range mg/Kg** | **Number of deaths** (**Male** / **Female**) 500: 0/5 / 0/5 800: 0/5 / 1/5 1300: 5/5 / 1/5 2000: 5/5 / 5/5	Anonymous 10 (1986) DAR, Spain, 2018
The study was designed to meet the requirements of the EPA 81-2 Guideline. Yes - Deviations from OECD 402 (1981): only two dose levels instead three dose-levels were used GLP	New Zealand White rabbits of both sexes (5 animals/sex/dose)	AGR 233011 - lot TB 860825-5), a liquid formulation with 52,63 wt% cis-1,3-Dichloropropene(Z) and 44,91 wt% trans-1,3,-D(E) (ML-AL 86-00703).	Undiluted test substance was applied at single dermal dose and concentrations of 200 and 1000 mg/kg bw to theback of the rabbits (5 animals by sex and dose) under occlusive dressing for 24 hours.	The acute dermal **LD_{50}** was **calculated for all animals as: 333 mg/kg** Dose range mg/Kg | Number of deaths (Male / Female) 200: 1/5 / 1/5 1000: 5/5 / 5/5	Anonymous 11 (1987) DAR, Spain, 2018
OECD 402 No deviations GLP	Sprague Dawley CFY strain of both sexes (5 rats/sex/group)	Cis 1,3-dichloropropene (Batch No. 87/RM/716) No information on purity is available in the study report.	All rats were treated with a single dermal dose of test material (occluded application) at doses of 500, 800, 1300 or 2000 mg/kg bw	**LD_{50} – All animals – 794** (669 – 942) **mg/kg** body weight. Males – 758 (604 – 950) mg/kg body weight Females – 841 (633 – 1118) mg/kg body weight **Dose range mg/Kg** | **Number of deaths** (**Male** / **Female**) 500: 0/5 / 0/5 800: 3/5 / 2/5 1300: 5/5 / 5/5 2000: 5/5 / 4/5	Anonymous 19 (1988)
EEC Method B3 No deviations GLP	Groups of five male and five female Fischer F344 rats	Trans-1,3-Dichloropropene Batch No. TR88001	488, 781, 1250 and 2000 mg/kg of undiluted test material	The acute (24 h) dermal **LD_{50} of the undiluted test material in rats was 1575 mg/kg** [95%	Anonymous 18 (1988)

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance,</th><th>Dose levels duration of exposure</th><th>Value
LD_{50}</th><th>Reference</th></tr>
<tr><td></td><td></td><td>Purity 96.7%</td><td></td><td>confidence interval 1163 to 3111 mg/kg].
<table><tr><th>Dose range mg/Kg</th><th colspan="2">Number of deaths</th></tr><tr><th></th><th>Male</th><th>Female</th></tr><tr><td>488</td><td>1/5</td><td>0/5</td></tr><tr><td>781</td><td>0/5</td><td>1/5</td></tr><tr><td>1250</td><td>1/5</td><td>2/5</td></tr><tr><td>2000</td><td>4/5</td><td>3/5</td></tr></table></td><td></td></tr>
<tr><td>OECD 402
No deviations
GLP</td><td>Groups of five male and five female Fischer F344 rats were dermally exposed to undiluted cis-1,3-dichloropropene at doses of 400, 560, 784, 1098 and 1537 mg/kg</td><td>Cis-1-3-Dichloropropene
Batch No. ST88/253
Purity 96.9%</td><td>400, 560, 784, 1098 and 1537 mg/kg</td><td>The acute (24-h) dermal LD_{50} of the undiluted test material in male rats was 1068 mg/kg (95% C.I. 722 to 3669 mg/kg), for female rats was 1190 mg/kg (95% C.I. 983 to 1315 mg/kg) and for combined sexes was 1090 mg/kg (95% C.I. 901 to 1403 mg/kg). Sites of application of cis-1,3-dichloropropene commonly showed erythema for up to four days after treatment
<table><tr><th>Dose range mg/Kg</th><th colspan="2">Number of deaths</th></tr><tr><th></th><th>Male</th><th>Female</th></tr><tr><td>400</td><td>1/5</td><td>0/5</td></tr><tr><td>560</td><td>0/5</td><td>0/5</td></tr><tr><td>784</td><td>2/5</td><td>0/5</td></tr><tr><td>1098</td><td>2/5</td><td>2/5</td></tr><tr><td>1537</td><td>4/5</td><td>5/5</td></tr></table></td><td>Anonymous 23 (1989)</td></tr>
</table>

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Acute toxicity of 1,3-D was investigated after dermal administration in rats, and rabbits. The acute dermal LD_{50} was calculated for rats as 1200 mg/kg bw, and separately for males as 1000 mg/kg bw and for females between 1300 and 2000 mg/kg bw (Anonymous 10, 1986). As regards other effects in rats, lethargy was observed in all animals on the day of dosing. Dose related signs of toxicity (decreased respiratory rate, increased lacrimation, increased salivation, ataxia, red/brown staining around the snout or mouth) were observed from 1300 mg/kg bw; signs of skin irritation manifested by edema, eschar formation or possible subcutaneous haemorrhage were seen at the test site. The signs of toxicity appeared earlier in animals treated with 2000 mg/kg bw, disappeared on day two in the animals treated with 500 mg/kg bw and on day three no systemic abnormalities in all of the surviving animals were noted. The body-weight gains of the majority of animals in this study were considered to be within normal limits. The abnormalities seen at necropsy of animals were associated with the lungs, liver, gastrointestinal tract and subcutaneous tissues at the site of application, they appeared haemorrhaged.

The acute dermal LD_{50} of 1,3-D was calculated for rabbits as 333 mg/kg bw (Anonymous 11, 1987). As regards other effects, some rabbits of both dose groups were restless and squealed shortly after treatment; all rabbits given 1000 mg/kg bw were lethargic prior to death. All animals had skin irritation (edema, erythema and necrosis) at the dermal test site. No significant effects on body weights were observed. At necropsy all rabbits had skin irritation with subcutaneous haemorrhage, necrosis, erythema, edema and/or crusts.

In the third acute dermal study (Anonymous 19, 1988), the cis-isomer of 1,3-dichloropropene was tested for acute percutaneous toxicity in groups of 10 rats (5 male and 5 female). Dose levels of 500-2000 mg/kg were applied beneath an occlusive dressing for a 24 hour exposure. Rats exposed to 500 mg/kg showed non-specific signs of intoxication lasting 2 days and severe skin damage but no deaths. Higher doses produced the same signs of intoxication and a dose related increase in mortality. Rats which died during the study had abnormalities of lungs, liver and gastrointestinal tract whereas survivors after 14 days were normal except for skin damage. The dermal LD_{50} of Cis-1,3 D was 758 mg/kg for males, 841 mg/kg for females and 794 mg/kg for the sexes combined (669 – 942 mg/kg 95% confidence level).

In the fourth study (Anonymous 18, 1988), groups of five male and five female Fischer F344 rats were used and exposed dermally to 488, 781, 1250 and 2000 mg/kg of undiluted trans-1,3-dichloropropene. On the day before dosing the dorsal fur was removed from the animals using electric clippers. Any rat showing signs of damage or irritation of the dorsum was replaced. On Day 1 the animals were weighed, and a single dose of the undiluted test material applied to the skin. The test material was held in place with a lint dressing (approx. 6 x 8 cm) covered with waterproof adhesive tape. The rats were then individually housed. Following a 24-hour exposure the dressings were removed, the skin washed with warm dilute detergent solution, dried, and the animals returned to group housing. A careful clinical examination was made three times daily for the first three days and once daily thereafter for the remainder of the 14-day observation period. The initial (Day 1), Day 7 and Day 14 bodyweights were recorded, and changes in bodyweight calculated. All animals were subject to necropsy. Animals sacrificed for humane reasons during the study or surviving to the end of the study were killed by an intraperitoneal injection of sodium pentobarbitone. The cranial, thoracic and abdominal cavities and viscera were examined and any gross pathological changes recorded.

The principal signs of reaction to treatment observed within five hours of topical application of trans-1,3-dichloropropene were voiding of soft faeces or diarrhoea, lethargy and, particularly at the higher dose levels, increased lachrymation. On the following day ataxia or abasia occurred among rats dosed at 1250 or 2000 mg/kg, voiding of soft faeces or diarrhoea persisted among those treated at 488 or 781 mg/kg and animals at all dose-levels showed an unkempt appearance and, less commonly, were prostrate. Two rats found with hind-limb immobility on Day 3 were sacrificed on humane grounds. Sites of application of the test substance generally showed erythema and oedema after removal of the dressings on Day 2. Erythema was the only common, persistent reaction to treatment observed from Day 3. All signs of reaction to treatment resolved by Day 7. Bodyweight losses between Days 1 and 7 were recorded for the majority of surviving rats. However, all of these animals gained weight relative to their Day 1 bodyweights by the end of the 14-day observation period.
The acute (24 h) dermal LD_{50} of the undiluted test material in rats was 1575 mg/kg [95% confidence interval 1163 to 3111 mg/kg].

In the last OECD Test Guideline 402 (GLP) study (Anonymous 23, 1989), groups of five male and five female Fischer F344 rats were dermally exposed to undiluted cis-1,3-dichloropropene at doses of 400, 560, 784, 1098 and 1537 mg/kg. On the day before dosing the dorsal fur was removed from the animals using electric clippers. Any rat showing signs of damage or irritation of the dorsum was replaced. On Day 1 the animals were weighed, and a single dose of the test material applied to the skin. The test material was held in place with a lint dressing (approx. 6x8 cm) covered with water-proof adhesive tape. The rats were then individually housed. Following a 24-hour exposure the dressings were removed, the skin washed with warm dilute detergent solution dried, and the animals returned to group housing. A careful clinical examination was made up to three times daily for the first three days and once daily thereafter for the remainder of the 14- day observation period. The initial Day 1, Day 7 and Day 14 bodyweights were recorded, and changes in bodyweight calculated. All animals were subject to necropsy. Animals sacrificed for humane reasons during the study or surviving to the end of the study were killed by an intraperitoneal injection of sodium pentobarbitone. The cranial, thoracic and

abdominal cavities and viscera were examined, and any gross pathological changes recorded. The 14-day LD50, 95% confidence interval and the dose-mortality slope were calculated using a method based on Probit analysis (Finney,1977).

Mortalities occurred on days 2 and 3. There were no clinical signs of systemic toxicity following administration of cis-1,3-dichloropropene at 400 mg/kg other than the death of a single male on Day 3. Voiding of soft faeces and lethargy were common among rats given higher dose levels and there were few additional cases of unkempt appearance, hypothermia, abasia, ataxia, hunched back or bloody discharge from the nose in the groups dosed at 784 mg/kg and above. Rats that died showed no ante-mortem clinical signs or similar responses to those of animals surviving treatment. The recovery of rats surviving treatment was generally advanced by Day 3 but remained incomplete until Day 6. Sites of application of the test compound commonly developed slight erythema or erythema that persisted for up to four days after removal of the dressings. Oedema was also apparent among rats treated at 1537 mg/kg. Various marks on or close to the dermal test sites were considered artefacts of preparation and bandaging of the dorsum. Despite loss of bodyweight or low bodyweight during the first week of the observation period, all surviving rats had gained weight relative to their Day 1 bodyweights at the end of the 14-day observation period. Necropsy of decedents commonly revealed abnormal contents, inflammation and haemorrhage of the stomach, discolouration of the subcutaneous muscle, discolouration of the renal medulla, lung congestion and petechiae on the thymus. No macroscopic abnormalities were found in rats that survived treatment.

The acute (24-h) dermal LD_{50} of the undiluted test material in male rats was 1068 mg/kg (95% C.I. 722 to 3669 mg/kg), for female rats was 1190 mg/kg (95% C.I. 983 to 1315 mg/kg) and for combined sexes was 1090 mg/kg (95% C.I. 901 to 1403 mg/kg). Sites of application of cis-1,3-dichloropropene commonly showed erythema for up to four days after treatment.

Common clinical symptoms were body weight loss, lethargy, diarrhoea, lacrimation, palpebral closure, laboured respiration, facial soiling, respiratory difficulty, skin irritation and/or rough hair coat. The treatment related gross changes were stomach lesions and lung congestion.

10.2.2 Comparison with the CLP criteria

In acute dermal toxicity studies conducted according to OECD TG 402 (or comparable methods i.e.: EEC Method B3), the LD_{50} values in rats were determined to be 1200 and 1575 mg/kg for 1,3-D (mix of isomers) and trans 1,3-D respectively (Anonymous 10 (1986), Anonymous 18 (1988)). In two respective rat studies using cis 1,3-D conducted in accordance with OECD TG 402, LD_{50} values of 794 and 1090 mg/kg bw were determined (Anonymous 19 (1988), Anonymous 23 (1989)). In an acute dermal toxicity study conducted in rabbits using 1,3-D (mix of isomers), the LD_{50} was determined to be 333 mg/kg bw/day (Anonymous 11 (1987)).

According to the criteria of the CLP Regulation: substances for which the acute dermal LD_{50} is > 1000 mg/kg and ≤ 2000 mg/kg bw require classification in Category 4 for acute dermal toxicity, whereas substances for which the acute dermal LD_{50} is > 200 and ≤ 1000 mg/kg bw require classification in Category 3 for acute dermal toxicity.

The evaluation of the available experimental data on 1,3-D (mix of isomers) and cis 1,3-D respectively indicates that these substances have a comparable profile of acute dermal toxicity and meet the CLP criteria for classification in Acute Toxicity Category 3 (H311) *"Toxic in contact with skin."* While the available data for trans 1,3-D meet the criteria for classification in Acute Toxicity Category 4 (H312) *"Harmful in contact with skin,"* the overall classification of the group of substances in Category 3 in respect of acute dermal toxicity would be considered to be precautionary.

Specific Concentration Limit of 333 mg/kg bw is proposed as the lowest acute dermal LD_{50} of the undiluted test material in male and female rabbits based on valid, well-performed study by Anonymous 11, 1987.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on data presented, 1,3-D (mix of isomers), (Z)1,3-D (cis-isomer) and (E)-1,3-D (trans- isomer) should be classified in accordance with the CLP Regulation as:

Acute toxicity Category 3, H311, Toxic in contact with skin with ATE value = 330 mg/kg bw (rounded-off to two significant figures).

10.3 Acute toxicity - inhalation route

Table 21: Summary table of animal studies on acute inhalation toxicity

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance, , form and particle size (MMAD)</th><th>Dose levels, duration of exposure</th><th>Value
LC_{50}</th><th>Reference</th></tr>
<tr>
<td>OECD 403 (1981), no deviations
GLP</td>
<td>Wistar rats, Groups of 5 Wistar albino rats by sex – whole body exposure</td>
<td>Test material: Telone II (Lot 0713054/062) a colourless liquid containing 98,4% 1,3-Dichloropropene.
No information on isomer composition is available in the study report, however cis- 1,3-dichloropropene content ≥45% is specified for Telone II</td>
<td>Whole-body exposure for 4 hours to vapour concentrations of 0, 1.62, 2.64, 2.70 or 3.07 mg/l.</td>
<td>The study estimated that the acute inhalatory LC_{50} lay between the exposure levels of 2.70 and 3.07 mg/L
<table>
<tr><th rowspan="2">Concentration mg/L</th><th colspan="2">Number of deaths</th></tr>
<tr><th>Male</th><th>Female</th></tr>
<tr><td>Control (air)</td><td>0/5</td><td>0/5</td></tr>
<tr><td>1.62</td><td>0/5</td><td>0/5</td></tr>
<tr><td>2.64</td><td>0/5</td><td>0/5</td></tr>
<tr><td>2.70</td><td>0/5</td><td>0/5</td></tr>
<tr><td>3.07</td><td>3/5</td><td>3/5</td></tr>
</table>
</td>
<td>Anonymous 12 (1987)
DAR, Spain, 2018</td>
</tr>
<tr>
<td>The Environmental Protection Agency (EPA), The Pesticide Assessment Guidelines and the Standard Operating Procedures
Deviations from OECD 403 (1981): none
GLP</td>
<td>Fisher 344 rats, Groups of 5 Fischer 344 rats/sex</td>
<td>Test material was Telone II soil fumigant (AGR 233011), a liquid containing 52.6% cis-1,3-Dichloropropene and 44.9% trans-1,3-Dichloropropene, lot TB 860825-5.</td>
<td>Whole-body exposed for 4 hours to vapours of Telone II soil fumigant at target concentrations of vapours 1000, 850, and 750 ppm[1], respectively</td>
<td>The inhalation LC_{50} in rats under the conditions of this study was between 3.88 and 4.70 mg/L for males and females.
<table>
<tr><th rowspan="2">Analytical Concen-tration (ppm)</th><th rowspan="2">Concen-tration (mg/L)</th><th colspan="2">Number of deaths</th></tr>
<tr><th>Male</th><th>Female</th></tr>
<tr><td>1035</td><td>4.70</td><td>5/5</td><td>5/5</td></tr>
<tr><td>855</td><td>3.88</td><td>0/5</td><td>1/5</td></tr>
<tr><td>904</td><td>4.10</td><td>0/5</td><td>0/5</td></tr>
<tr><td colspan="4">LC_{50} for males between 3.88-4.70-mg/LLC_{50} for females is 4.10mg/L</td></tr>
</table>
</td>
<td>Anonymous 13 (1987)
DAR, Spain, July 2018</td>
</tr>
</table>

[1] According to NIOSH Pocket Guide to Chemical Hazard (http://www.cdc.gov/niosh/npg/npgd0199.html), the conversion of ppm (parts of vapour or gas per million parts of contaminated air by volume) to mg/m3(milligrams of vapour or gas per cubic meter of contaminated air) at 25°C and 1 atmosphere for 1,3-Dichloropropene is 1ppm = 4.54 mg/m3

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance, , form and particle size (MMAD)</th><th>Dose levels, duration of exposure</th><th>Value
LC_{50}</th><th>Reference</th></tr>
<tr><td>OECD 403 (1983), GLP – not stated</td><td>Fischer 344 rats – 5 male/dose group</td><td>TELONE II Soil Fumigant (Lot 121006-9). The liquid test material contained 97.5% 1,3-dichloropropene (cis and trans) as active ingredients.

No information on isomer composition is available in the study report, however cis- 1,3-dichloropropene content ≥45% is specified for Telone II</td><td>Nose-only exposed to a nominal concentration of 14,980 ppm TELONE II vapours for 1-hour</td><td>One-hour inhalation exposure to 14,000 ppm (analytical concentration) TELONE II caused mortality in all exposed Fischer 344 rats. (using the Haber's Law and conversion[1] – 1 ppm =4.54 mg/m^3), this translates to approx. 15.90 mg/l)</td><td>Anonymous 142 (2003); DAR, Spain, 2018</td></tr>
<tr><td>OECD Guideline 403, EPA Guideline 81-3, no deviations, GLP</td><td>Groups of 5 male and 5 female Fischer 344 rats were exposed (vapour, whole body under dynamic airflow conditions) to 573, 771 and 1020 ppm of the test material for a single 4-hour period.</td><td>The test material consisted of 95.6% cis 1,3-Dichloropropene, 1.5% trans 1,3-Dichloropropene, and 0.2% 1,2-Dichloropropane</td><td>Whole body under dynamic airflow conditions) to 573, 771 and 1020 ppm of the test material for a single 4-hour period.</td><td>The LC50 for male and female rats was 670 and 744 ppm (3.0 and 3.4 mg/L) cis-1,3-dichloro-propene, respectively
<table>
<tr><th rowspan="2">Analytical Concen-tration (ppm)</th><th rowspan="2">Concen-tration (mg/L)</th><th colspan="2">Number of deaths</th></tr>
<tr><th>Male</th><th>Female</th></tr>
<tr><td>573</td><td>2.60</td><td>0/5</td><td>0/5</td></tr>
<tr><td>771</td><td>3.50</td><td>5/5</td><td>3/5</td></tr>
<tr><td>1020</td><td>4.63</td><td>5/5</td><td>5/5</td></tr>
<tr><td colspan="4">LC_{50} for males between 2.60-3.50 mg/L, for females 3.50 – 4.63 mg/L</td></tr>
</table></td><td>Anonymous 20 (1990)</td></tr>
<tr><td>OECD Guideline 403 No deviations GLP</td><td>Groups of 5 male and 5 female rats (Crl:CD(SD)BR) were exposed to (head only) exposure under dynamic continuous flow system) to aerosol concentrations of 850, 980, 1097 and 1401 ppm (3.859, 4.449, 4.98 and 6.36 mg/l), over a period of 4 hours, a similar</td><td>The test material was trans-1,3-dichloropropene (Lot no. ST 88/098), purity – 96.7%</td><td>Head only exposure under dynamic continuous flow system) to aerosol concentrations of 850, 980, 1097 and 1401 ppm (3.859, 4.449, 4.98 and 6.36 mg/l), over a period of 4 hours</td><td>The acute median lethal concentration and 95% fiducial limit, calculated by a probit method were: sexes combined 1123 ppm – 5.098 mg/l, males only 1190 ppm – 5.403 mg/l (limits not calculable) females only 1075 ppm – 4.881 mg/l
<table>
<tr><th rowspan="2">Concentration mg/L</th><th colspan="2">Number of deaths</th></tr>
<tr><th>Male</th><th>Female</th></tr>
<tr><td>3.859</td><td>1/5</td><td>1/5</td></tr>
<tr><td>4.449</td><td>1/5</td><td>0/5</td></tr>
<tr><td>4.98</td><td>3/5</td><td>3/5</td></tr>
<tr><td>6.36</td><td>3/5</td><td>5/5</td></tr>
</table></td><td>Anonymous 21 (1989)</td></tr>
</table>

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
	group of rats were exposed to filtered air, served as control				

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

Acute toxicity of 1,3-D was investigated after inhalation exposure to vapour of 1,3-D in rats. In Wistar albino rats (Anonymous 12, 1987) the inhalation LC_{50} was between 2.70 and 3.07 mg/L. No animals died up to 2.70 mg/L. During the exposure to the test substance, all animals showed partial closing of the eyes, lacrimation, reduce respiratory rate and irregular respiratory movements. At the observation period, hunched posture and restless behaviour were seen in the majority of exposed rats. Clinical signs persisted in a proportion of rats for several days following exposure (lethargy, reduced respiratory rate, shallow respiratory movements, irregular respiratory movements, diarrhoea, brown staining of the fur and fur loss). The body weights and food and water consumption of survivors were reduced for up to 6 days following exposure. Finding of necropsy of surviving rats were associated fundamentally with the lungs and respiratory tract, gastrointestinal tract and adrenals.

In Fischer 344 rats (Anonymous 13, 1987), inhalation LC_{50} was between 3.88 and 4.70 mg/L for males and 4.10 mg/L for females. No rats died at the lowest dose, but all animals showed signs of irritation and were lethargic at all dose levels during and after exposure. Body weights of survivors decreased during the first week post-exposure and increased during the second week. Animals which died as a result of exposure had facial soiling and/or haemorrhages in multiple lung lobes. Surviving animals did not have any exposure-related gross observations.

A group of five male Fischer 344 (Anonymous 142, 2003) rats was nose-only exposed to a nominal concentration of 14980 ppm TELONE II vapours for 1-hour. Test atmosphere concentration from the breathing zone of the animals, exposure room temperature, chamber temperature, humidity and air flow were recorded every 10 minutes. Clinical observations and gross necropsy were evaluated on all animals.

Table 22: Summary of inhalation exposure parameters to Telone II soil fumigant

Nominal concentration (ppm)	Analytical concentration (ppm)	Mean temperature (°C)	Mean RH (%)	Mean chamber air flow (L/min)
14980	14000 ± 171	22.8 ± 0.8	45.9 ± 0.38	30

All animals survived the one-hour exposure to the test material, one rat was found dead approximately one hour post-exposure; the remaining rats were found dead at the morning cageside observation on test day 2. Clinical effects noted during the one-hour exposure period included perinasal soiling and slow, labored breathing in all animals. Post-exposure observations on test day 1 included mortality; inability to walk; slow, shallow, noisy, labored, and/or deep respiration; perinasal, perineal, and/or abdominal soiling; bluish skin and mucous membranes; and partially closed eyelids. Decreases in resistance to removal, muscle tone, extensor-thrust response, reactivity to stimuli and responsiveness to touch were also observed post-exposure on test day 1. Necropsy findings included mottled lungs and serosanguineous soiling of the muzzle in all animals, and multifocal ulcers of the stomach in one animal.

One-hour inhalation exposure to 14,000 ppm TELONE II caused mortality in all exposed Fischer 344 rats. Using the Habers Law conversion (to 4 h exposure) in Study 2 – 1 ppm – 4.54 mg/m3, this translates to approx. 15.90 mg/l,

Four groups of ten rats (five males and five females) of the Crl:CD(SD)BR strain were exposed to trans-1,3-dichloropropene at single chamber concentrations of 850, 980, 1097 and 1401 ppm (3.859, 4.449, 4.980 and 6.360 mg/1) by inhalation (aerosol, head-only) over a period of four hours (Anonymous 21, 1989). Corresponding nominal concentrations were in the range 755 to 1318 ppm. A similar group of ten rats (five males and five females) was exposed to filtered air as a control. Exposure was followed by an observation period of 14 days. 1.2 The exposure chamber temperature recorded for the control and treated groups ranged between 17 and 21°C, and the chamber relative humidity was between 23 and 52%. 1.3 The chamber air flow was between 17 and 21 1/min for the control and test groups. 1.4 Deaths occurred in all treated groups between days 2 and 8. The incidence of deaths correlated with atmosphere concentration. 1.5 Marked treatment-related clinical signs occurred at all concentrations and included piloerection, salivation, coldness to touch, hunched posture, lethargy and stained fur. 1.6 Animals from all treated groups showed an adverse effect on body weight. The majority of treated animals regained their pre-exposure body weight by day 15. 1.7 Decedents showed marked increases in lung weights which may have been treatment-related or due only to agonal changes. There were no changes in lung weights in animals surviving to termination.

Most survivors were comparable with controls at necropsy. Animals that died typically showed pathological signs of cardiopulmonary failure, acute tubular necrosis of the inner renal cortex and, in a few individuals. local effects on the respiratory tract. Recent renal tubular regeneration was observed in the kidneys of survivors at termination.

The acute median lethal concentration and 95% fiducial limit, calculated by a probit method were: sexes combined 1123 ppm – 5.098 mg/l (1000 to 1342 ppm – 4.54 – 6.092 mg/l), males only 1190 ppm (limits not calculable) females only 1075 ppm – 4.881 mg/l (945 to 1313 ppm – 4.290 – 5.961 mg/l).

An acute inhalation study (Anonymous 20, 1990) was conducted to determine the LC_{50} for cis-1,3-dichloropropene (cis-DCP). Groups of 5 male and 5 female Fischer 344 rats were exposed to analytically measured concentrations of 583, 771 and 1020 ppm cis-DCP for 4- hours under dynamic airflow conditions. Animals were observed daily and were weighed on test days 1, 2, 4, 8, 11 and 15. All dead animals were necropsied; survivors were necropsied on test day 15. All animals died during or shortly after exposure to 1020 ppm cis-DCP. Five male and three female rats exposed to 771ppm died during the exposure or during the two-week observation period. All rats survived the 4-hour exposure to 583 ppm as well as the two-week post-exposure period. All clinical observations were transitory and limited to eyes and respiratory tract. Bodyweights for male and female rats exposed to 583 ppm decreased 11-13% on test day 2 from pre-exposure values. These animals subsequently started to gain weight by test day 8. Gross pathologic effects in animals dying during the exposure or observation period were confined to the eye and respiratory tract. There were no grossly visible lesions noted at the end of the two-week observation period in the two females exposed to 771 ppm or in any of the animals exposed to 583 ppm cis-DCP. The LC_{50} for male and female rats was 670 and 744ppm cis-1,3-dichloropropene, respectively.Common clinical symptoms were body weight loss, lethargy, diarrhoea, lacrimation, palpebral closure, laboured respiration, facial soiling, respiratory difficulty, skin irritation and/or rough hair coat. The treatment related gross changes were stomach lesions and lung congestion.

10.3.2 Comparison with the CLP criteria

In two acute inhalation toxicity studies conducted according to OECD TG 403 in which rats received whole body exposures to vapours of 1,3-D (mix of isomers), the 4-hour inhalation LC_{50} values ranged from 2.70-3.07 mg/L and from 3.88-4.70 mg//L respectively (Anonymous 12 (1987), Anonymous 13 (1987)).

In an acute inhalation toxicity study conducted according to EPA Guideline 81-3 in which rats received whole body exposures to cis 1,3-D, the 4-hour inhalation to vapour LC_{50} values were determined to be 670 ppm (3.04 mg/L) and 744 ppm (3.38 mg/L) in male and female rats respectively (Anonymous 20 (1990)).

In an acute inhalation toxicity study conducted according to OECD TG 403 in which rats received head only exposures to aerosol (as described in the study report) of trans 1,3-D, the 4-hour inhalation LC_{50} value was determined to be 5.098 (4.54-6.092) mg/L (both sexes), (Anonymous 21 (1989)).

According to the criteria of the CLP Regulation: substances for which the acute inhalation 4-hour LC_{50} (vapours) > 2 and ≤ 10 mg/kg bw require classification in Acute Toxicity Category 3 (H331) *"Toxic if inhaled."*

The evaluation of available experimental data on 1,3-D (mix of isomers), cis 1,3-D and trans 1,3-D indicates that these substances have a comparable profile with respect to the potential for acute inhalation toxicity. These substances respectively meet the CLP criteria for classification in Acute Toxicity Category 3 (H331) "*Toxic if inhaled."*

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on data presented, 1,3-D (mix of isomers), (Z)-1,3-D (cis isomer) and (E)-1,3-D (trans isomer) should be classified in accordance with the CLP Regulation as:
Acute toxicity Category 3, H331, Toxic if inhaled. No harmonised ATE-value is proposed.

10.4 Skin corrosion/irritation

Table 23: Summary table of animal studies on skin corrosion/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
EPA 81-5, no GLP	Six (two male and four female) New Zealand White rabbits were used	The test material was 1,3-dichloropropene (AGR 233011), lot TB 860825-5 containing 52.63% cis isomer and 44.91% trans isomer	Aliquots of 0.5 mL of the undiluted test material was applied to the shaved back of the animals under semi occlusive dressing for four hours	Immediately following the four-hour exposure period, the application sites had slight to moderate erythema and moderate to severe oedema, which persisted in two of six rabbits 14 days post-treatment. One rabbit had no signs of dermal irritation at study termination. Four rabbits were observed with exfoliation at 7-day and 14 day observation period. The mean score for erythema of each tested animal from gradings at 24, 48 and 72 hours after patch removal were: 2.0, 2.0, 1.66, 2.66, 2.66, 2.33. The mean score for oedema of each tested animal from gradings at 24, 48 and 72 hours after patch removal were: 1.0, 1.66, 1.33, 1.33, 1.66, 2.33. Inflammation persisted to the end of the observation period of 14 days in 4 animals.	Anonymous 14 (1987) DAR, Spain, 2018
EEC Method B4 No deviations	Six New Zealand White rabbits were used (3 males +	Trans-1,3-Dichloropropene Batch No. TR88001 Purity 96.7%	0.5 ml of undiluted test material	In the 4-hour rabbit skin irritancy test the test material caused irritation reactions not exceeding well-defined erythema and slight oedema. Resolution of the irritation responses was first apparent 72 hours	Anonymous 18 (1988)

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
GLP	3 females)			after treatment and was complete by the 21st day of the observation period.	
OECD 404 No deviations GLP	Six New Zealand White rabbits were used	Cis-1-3-Dichloropropene Batch No. ST88/253 Purity 96.9%	0.5 ml of undiluted test material	In the 4-hour rabbit skin irritancy test the test material caused well-defined erythema and moderate oedema at all dermal test sites shortly after removal of the semi-occlusive dressings. Resolution of the irritation reactions was first apparent on the day after treatment and was complete within 14 days	Anonymous 23 (1989)

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

In an EPA 81-5 guideline study (Anonymous 14, 1987), 1,3-D was tested for skin irritation in New Zealand White rabbits. Aliquots of 0,5 mL of the undiluted test material was applied to the shaved back of the animals under semi-occlusive dressing for four hours. Immediately following the four-hour exposure period, the application sites had slight to moderate erythema and moderate to severe oedema, which persisted in two of six rabbits 14 days post-treatment. One rabbit had no signs of dermal irritation at study termination. Four rabbits were observed with exfoliation at 7-day and 14 day observation period. The mean score for erythema of each tested animal from gradings at 24, 48 and 72 hours after patch removal were 2.0, 2.0, 1.66, 2.66, 2.66, 2.33. The mean score for oedema of each tested animal from gradings at 24, 48 and 72 hours were 1.0, 1.66, 1.33, 1.33, 1.66, 2.33. Inflammation persisted to the end of the observation period of 14 days in 4 animals with erythema score 2, oedema score from 1 to 2 and exfoliation.

In the second study (trans 1,3-dichloropropene), six New Zealand White rabbits were used. On the day before dosing the dorsal fur was removed using electric clippers. Any rabbit with signs of damage or irritation of the dorsum was replaced. The undiluted test material (0.5 ml) was applied to the skin on a 6 cm^2 lint patch, covered with gauze and held in place by a semi-occlusive elastic adhesive bandage. After a 4-hour exposure the dressings were removed, the skin washed with water and dried. After treatment the animals were examined for erythema, oedema and other lesions. Erythema and oedema were each scored on a four-point scale. The mean scores at each time point and group mean scores at 24, 48 and 72 hours were calculated.

Table 24: Mean Dermal irritation following a 4-hour application to rabbit skin of trans-1,3-dichlororpropene

Group mean of 6 rabbits (3 males + 3 females) – 24, 48 and 72-hour scores	
Erythema	1.7
Edema	0.8

Application of 0.5 ml undiluted trans-1,3-dichloropropene to the clipped dorsal skin of six rabbits caused irritation reactions not exceeding well-defined erythema and slight oedema. Such responses were first observed one-half hour after removal of the occlusive dressings. Resolution of irritation reactions was first apparent 72 hours after treatment and was complete by the 21st day of the observation period.
In the 4-hour rabbit skin irritancy test the test material caused irritation reactions not exceeding well-defined erythema and slight oedema. Resolution of the irritation responses was first apparent 72 hours after treatment and was complete by the 21st day of the observation period.

In the last OECD guideline 404 (GLP) study using cis 1,3-dichloropropene, (4-hour rabbit skin irritancy test), six rabbits were used. On the day before dosing the dorsal fur was removed using electric clippers. Any rabbit

with signs of damage or irritation of the dorsum was replaced. The undiluted test material (0.5 ml) was applied to the skin on a 6 cm2 lint patch, covered with gauze and held in place by a semi-occlusive elastic adhesive bandage. After a 4-hour exposure the dressings were removed, the skin washed with water and dried. After treatment the animals were examined for erythema, oedema and other lesions. Erythema and oedema were each scored on a four-point scale. The mean scores at each time point and group mean scores at 24, 48 and 72 hours were calculated.

Application of 0.5 ml of undiluted cis-1,3-dichloropropene to the clipped dorsal skin of six rabbits caused well-defined erythema and moderate oedema at all dermal test sites one-half hour after removal of the semi-occlusive dressings. Subsequent examinations revealed progressive resolution of the irritation reactions. All erythema and oedema resolved by the fourteenth day of the observation period. Desquamation of the treated skin was apparent in all rabbits on Days 7 and14 but persisted in only one animal on Day 21.
In the 4-hour rabbit skin irritancy test the test material caused well-defined erythema and moderate oedema at all dermal test sites shortly after removal of the semi-occlusive dressings. Resolution of the irritation reactions was first apparent on the day after treatment and was complete within 14 days.

10.4.2 Comparison with the CLP criteria

In an investigation of the skin irritation potential of 1,3-D (mix of isomers) in NZW rabbits according to EPA Guideline 81-5 (Anonymous 14 (1987)), the mean scores for erythema of each tested animal from gradings at 24, 48 and 72 hours after patch removal were 2.0, 2.0, 1.66, 2.66, 2.66 and 2.33. The mean scores for oedema of each tested animal from gradings at 24, 48 and 72 hours were 1.0, 1.66, 1.33, 1.33, 1.66 and 2.33. Inflammation persisted to the end of the observation period of 14 days in 4 animals with erythema score 2, oedema score from 1 to 2 and exfoliation. The criterion for classification (CLP Regulation) in Category 2 of skin irritation: "mean score of ≥ 2.3 and ≤ 4.0 for erythema/ eschar or for oedema in at least 2 (4) of 3 (6) i.e.:66.7% of tested animals from gradings at 24, 48 and 72 hours after patch removal" has not been met for 1,3-D. However, the criterion: "Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals" has been met. Hence, on the basis of these findings, 1,3-D (mix of isomers) meets the CLP criteria for classification in Skin irritation Category 2 (H315) *"Causes skin irritation."*

In an investigation of the skin irritation potential of cis 1,3-D (cis isomer) in NZW rabbits according to OECD Test Guideline 404 (Anonymous 23 (1989)), the mean scores for erythema of each tested animal from gradings at 24, 48 and 72 hours after patch removal were 1.0, 1.0, 1.33, 1.33, 1.67 and 1.33. The mean scores for oedema of each tested animal from gradings at 24, 48 and 72 hours were 1.33, 1.67, 2, 1.67, 1.0 and 1.33. Dermal desquamation of the treated area was observed in all animals at 7 and at 14 days after treatment and persisted in one animal until day 21. The criterion for classification (CLP Regulation) in Category 2 of skin irritation: "mean score of ≥ 2.3 and ≤ 4.0 for erythema/ eschar or for oedema in at least 2 (4) of 3 (6) i.e.: 66.7% of tested animals from gradings at 24, 48 and 72 hours after patch removal" has not been met for cis 1,3-D. However, the criterion: "Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals" is considered to be met, on the basis that dermal desquamation, an effect relevant to the consideration of persistent inflammation for classification purposes was observed in all animals at 7 and at 14 days after treatment. Hence, on the basis of these findings, cis 1,3-D meets the CLP criteria for classification in Skin Irritation Category 2 (H315) *"Causes skin irritation."*

In an investigation of the skin irritation potential of trans 1,3-D (trans isomer) in NZW rabbits according to EEC Method B4 (Anonymous 18 (1988), the mean scores for erythema of each tested animal from gradings at 24, 48 and 72 hours after patch removal were 1.67, 1.67, 1.67, 2.0, 2.0 and 1.0. The mean scores for oedema of each tested animal from gradings at 24, 48 and 72 hours were 0.67, 0.67, 0.67, 1.0, 1.0 and 0.33. Dermal desquamation of the treated area was observed in two animals with mean scores for erythema of 1.67 and 2.0 respectively, 14 days after treatment. The criterion for classification (CLP Regulation) in Category 2 for skin irritation: "mean score of ≥ 2.3 and ≤ 4.0 for erythema/ eschar or for oedema in at least 2 (4) of 3 (6) i.e.: 66.7% of tested animals from gradings at 24, 48 and 72 hours after patch removal" has not been met for trans 1,3-D. However, the criterion: "Inflammation that persists to the end of the observation period normally 14

days in at least 2 animals" is considered to be met, on the basis that dermal desquamation, an effect relevant to the consideration of persistent inflammation for classification purposes was observed in two animals with means scores of 1.67 and 2.0 for erythema, 14 days after treatment. Hence, on the basis of these findings, trans 1,3-D meets the CLP criteria for classification in Skin Irritation Category 2 (H315) *"Causes skin irritation."*

Based on the evaluation of available experimental data, 1,3-D (mix of isomers), cis 1,3-D and trans 1,3-D have a comparable profile with respect to the potential for skin irritation. These substances respectively meet the CLP criteria for classification in Skin Irritation Category 2 (H315) *"Causes skin irritation."*

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Based on the data presented, 1,3-D (mix of isomers), (Z)-1,3-D (cis isomer) and (E)--1,3-D (trans isomer) should be classified in accordance with the CLP Regulation as:

Skin Irritation Category 2, H315, Causes skin irritation.

10.5 Serious eye damage/eye irritation

Table 25: 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**
EPA 81-4 GLP	Four male and two female New Zealand White rabbits were used	The test material was AGR 233011, lot TB 860825-5 containing 52.63% cis isomer and 44.91% trans isomer 1,3-dichloropropene	Aliquots 0.1 mL of the undiluted test material were instilled into the conjunctival sac of the right eye of each animal. The left eye remained untreated and served as control. Because a moderate discomfort was observed in the first animal upon instillation of the test material, therefore, the treated eyes of the remaining animals were anaesthetized. Both eyes of the rabbits were examined with a penlight at 1, 24, 48 and 72 hours and 7 and 14 days. The eyes remained unwashed. Irritation was scored according to Draize method.	The eyes examination revealed slight to marked redness and light to moderate chemosis. A slight to moderate amount of discharge was present in treated eyes. Five rabbits had reddening of the iris and one rabbit had scattered or diffuse areas of corneal opacity, that was resolved within14 days post-treatment. The mean scores following grading at 24, 48 and 72 hours after instillation of the test material for: corneal opacity- 0.0, 0.0, 1.0, 0.0, 0.0, 0.0, iritis- 1.0, 0.0, 1.0, 0.33, 1.0, 0.0 conjunctival redness- **2.66**, 0.66, **3.0, 2.0, 3.0,** 1.66 conjunctival oedema- 2.66, 0.0, 2.66, 1.0, 2.0, 1.0 All signs of eye irritation gradually subsided and were absent 14 days post-treatment. Under the conditions of this study , since mean scores of conjunctival redness ≥ 2 were found in 4 of 6 tested animals, 1,3-D caused ocular	Anonymous 15 (1987) DAR, Spain, 2018

				irritation and would be classified as “Irritating to eyes" Category 2, H319.	
EEC Method B5 No deviations GLP	Six New Zealand White rabbits (3 males + 3 females) with eyes free from irritation, ocular defects or corneal injury were used	Trans-1,3-Dichloropropene Batch No. TR88001 Purity 96.7%	The undiluted test material (0.1 ml) was placed into the lower conjunctival sac of one eye of each animal. The treated eye was gently held closed for a few seconds to prevent loss of the test material. The eyes were not irrigated. The immediate reactions of the rabbit were scored as an initial pain response using a six-point scale. Other ocular reactions to treatment were noted and scored using standard grades. The mean scores at each time point and the group mean scores at 24, 48 and 72 hours were calculated.	In the rabbit eye irritancy test the test material (0.1 ml) commonly caused moderate to severe conjunctival irritation reactions and minor irritation changes of the cornea and iris within 24 hours of instillation into the eye. Resolution of the irritant effects of trans-1,3-dichloropropene was advanced seven days after treatment and complete one week later. Upon administration of the test material there was a severe initial pain response.	Anonymous 18 (1988)
Isolated Eye Test (Price and Andrews, 1985) No deviations GLP	Three New Zealand White rabbits were killed by intravenous injection of sodium pentobarbitone and their eyes excised.	Cis-1-3-Dichloropropene Batch No. ST88/253 Purity 96.9%	0.1 ml of undiluted cis-1,3-dichloropropene was applied to each eye	The corneal thickness of isolated eye preparations subject to application of cis-1,3-dichloropropene increased by more than 20% within 3 hours of treatment. Corneal uptake of fluorescein was demonstrated at the conclusion of the test. The result indicated that application of cis-1,3-dichloropropene to the eye in vivo would cause significant tissue damage	Anonymous 23 (1989)

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

In an EPA 81-4 guideline study, 1,3-D was tested for ocular irritation in four male and two female New Zealand White rabbits. Aliquots 0.1 mL of the undiluted test material were instilled into the conjunctival sac of the right eye of each animal. The left eye remained untreated and served as control. Because a moderate discomfort was observed in the first animal upon instillation of the test material, therefore, the treated eyes of the remaining animals were anaesthetized. Both eyes of the rabbits were examined with a penlight at 1, 24, 48 and 72 hours and 7 and 14 days. The eyes remained unwashed. Irritation was scored according to Draize method.

The eyes examination revealed slight to marked redness and light to moderate chemosis. A slight to moderate amount of discharge was present in treated eyes. Five rabbits had reddening of the iris and one rabbit had scattered or diffuse areas of corneal opacity, that was resolved within14 days post-treatment. All signs of eye irritation gradually subsided and were absent 14 days post-treatment.

In the second study conducted using trans 1,3-dichloropropene according to EEC Method B5, six New Zealand White rabbits with eyes free from irritation, ocular defects or corneal injury were used. The undiluted test material (0.1 ml) was placed into the lower conjunctival sac of one eye of each animal. The treated eye was gently held closed for a few seconds to prevent loss of the test material. The eyes were not irrigated. The immediate reactions of the rabbit were scored as an initial pain response using a six-point scale. Other ocular reactions to treatment were noted and scored using standard grades. The mean scores at each time point and the group mean scores at 24, 48 and 72 hours were calculated.

Table 26: Mean Eye Irritation Scores following the instillation of trans-1,3-Dichloropropene into rabbit eyes

Group Mean (6 rabbits – 3 males + 3 females) – 24, 48 and 72-hour scores	
Redness	2.1
Chemosis	1.2
Corneal opacity	0.7
Iritic effects	0.1

Instillation of 0.1 ml undiluted trans-1,3-dichloropropene into the eyes of six rabbits resulted in severe initial pain responses.

Conjunctival irritation was apparent in all rabbits one hour after ocular instillation of the test material. Minor corneal opacities developed in five rabbits and there were iridial responses in three of the same animals within 24 hours of treatment. Maximum irritation responses, observed between 4 and 24 hours after treatment, were a beefy-red appearance of the conjunctivae, chemosis obscuring up to one-half of the eye, diffuse opacity of the cornea and congestion of the iris. Resolution of the irritant effects of trans-1,3-dichloropropene was advanced seven days after treatment and complete one week later.

In the rabbit eye irritancy test the test material (0.1 ml) commonly caused moderate to severe conjunctival irritation reactions and minor irritation changes of the cornea and iris within 24 hours of instillation into the eye. Resolution of the irritant effects of trans-1,3-dichloropropene was advanced seven days after treatment and complete one week later. Upon administration of the test material there was a severe initial pain response.

In the third study, the eye irritancy potential of cis-1,3-dichloropropene was assessed using the Isolated Eye Test (Price and Andrews, 1985). Three rabbits were killed by intravenous injection of sodium pentobarbitone and their eyes excised. The eyes were held in clamps designed to secure the eye in the desired orientation without deformation. Each clamp plus eye was mounted in a chamber held at approximately 32°C by means of a circulating water jacket. The eyes were superfused with warm saline which dripped across the frontal surface of the eye. After one hour's equilibration, the corneal thickness of the excised eyes was measured using a pachymeter attachment to a Zeiss photo-slit lamp ophthalmic microscope and the values compared with previously determined in vivo values. The in vivo and ex vivo values showed-no significant differences and there was no corneal staining upon application of fluorescein, confirming that the eyes had not been damaged during the dissection procedure. The saline drip was diverted and 0.1 ml of undiluted cis-1,3-dichloropropene was applied to each eye. After about 10 seconds each eye was irrigated with approximately 5 ml of warm saline. The corneal thickness of each eye was measured at hourly intervals after dosing for a total of 5 hours. After the 4-hour reading, fluorescein was applied to each eye and the degree of staining noted. The mean corneal thickness at each observation time was calculated and used to grade the eye irritancy potential of the test material.

Application of 0.1 ml undiluted cis-1,3-dichloropropene to each of six isolated eye preparations resulted in increase of corneal thickness by more than 20% (group mean value) within 3-hours of treatment. Corneal uptake of fluorescein was apparent in all preparations following application of the dye to the corneal surface 4-hours after treatment. Based on these results, cis-1,3-dichloropropene was classified as a Grade IV irritant.

It is predicted that application of the test compound to the eye, in vivo, would result in significant ocular damage persisting 21 days after treatment.

The corneal thickness of isolated eye preparations subject to application of cis-1,3-dichloropropene increased by more than 20% within 3 hours of treatment. Corneal uptake of fluorescein was demonstrated at the conclusion of the test. The result indicated that application of cis-1,3-dichloropropene to the eye in vivo would cause significant tissue damage.

10.5.2 Comparison with the CLP criteria

In an investigation of the eye irritation potential of 1,3-D (mix of isomers) in NZW rabbits conducted according to EPA Guideline 81-4 (Anonymous 15 (1987)), the mean scores following grading at 24, 48 and 72 hours after instillation of the test material were: 0.0, 0.0, 1.0, 0.0, 0.0 and 0.0 for corneal opacity; 1.0, 0.0, 1.0, 0.33, 1.0 and 0.0 for iritis; 2.66, 0.66, 3.0, 2.0, 3.0 and 1.66 for conjunctival redness and 2.66, 0.0, 2.66, 1.0, 2.0 and 1.0 conjunctival oedema (chemosis). All signs of eye irritation gradually subsided and were absent 14 days post-treatment. The criterion for classification (CLP Regulation) in Category 2 for eye irritation: "conjunctival redness ≥ 2.0" in 2/3 (i.e.: 66.7%) animals from gradings at 24, 48 and 72 hours after treatment which fully reversed within 21 days of treatment" was met. Hence, on the basis of this study,1,3-D (mix of isomers) meets the CLP criteria for classification in Eye Irritation Category 2 (H319) *"Causes serious eye irritation."*

In an investigation of the eye irritation potential of cis 1,3-D in the excised eyes of NZW rabbits in an Isolated Eye Test, the application of 0.1 mL of undiluted cis 1,3-D to each of six isolated rabbit eye preparations resulted in an increase of corneal thickness by more than 20% (group mean value) within 3 hours of treatment (Anonymous 23 (1989)). Corneal uptake of fluorescein was apparent in all preparations following application of the dye to the corneal surface 4 hours after treatment. On the basis of these results *in vitro*, the application of cis 1,3-D *in vivo* is predicted to result in significant ocular damage persisting for 21 days after treatment.

In an investigation of the eye irritation potential of trans 1,3-D in NZW rabbits conducted according to EEC Method B5 (Anonymous 18 (1988)), the mean scores following grading at 24, 48 and 72 hours after instillation of the test material were: 1.0, 0.67, 0.67, 0, 0.67 and 1.0 for corneal opacity; 0, 0, 0.33, 0, 0.33 and 0 for iritis; 3.0, 1.67, 1.67, 1.67, 2.0 and 2.67 for conjunctival redness and 1.67, 1.0, 1.0, 1.0, 1.33 and 1.33 for conjunctival oedema (chemosis). Resolution of the eye irritant effects was advanced 7 days after treatment and fully resolved within 14 days. The criteria for classification (CLP Regulation) in Category 2 for the eye irritation effects: corneal opacity, iritis, conjunctival redness and conjunctival oedema (chemosis) were not met based on gradings at 24, 48 and 72 hours after treatment and irritation effects fully reversed within 21 days of treatment. On the basis of this study, trans 1,3-D does not meet the CLP criteria for classification in respect of eye irritation.

According to the Guidance on the Application of the CLP Criteria (Version 5.0, July 2017), the Isolated Rabbit Eye (IRE) *ex vivo* test can be regarded as a validated test method without an OECD Test Guideline, and positive findings from the test can be taken into consideration as part of a "Top-Down" precautionary approach for the classification of substances in serious eye damage Category 1 for example, when no other data are available and for the avoidance of further animal testing, if deemed unnecessary. Since *in vivo*, guideline studies are available for 1,3-D a weight of evidence approach is considered to be appropriate. Overall, 1,3-D (mix of isomers) and cis 1,3-D have demonstratable eye irritation potential whereas trans 1,3-D causes mild irritation effects that do not meet the criteria for classification, hence classification of the group overall as Eye Irritation Category 2 (H319) *"Causes serious eye irritation,"* is considered to be appropriate.

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

Based on data presented, 1,3-D (mix of isomers), (Z)-1,3-D (cis-isomers) and (E)-1,3-D (trans-isomers) should be classified in accordance with the CLP Regulation as:

Eye Irritation Category 2, H319, Causes serious eye irritation.

10.6 Respiratory sensitisation

Not assessed in this dossier.

10.7 Skin sensitisation

Table 27: Summary table of animal studies on skin sensitisation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance,</th><th>Dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>OECD 406. Buehler test
Deviations: It is not indicated if Guinea pigs had received an adequate amount of ascorbic acid. A modified Buehler method was used: the number of animals used (10 in treatment group) was lower than that recommended for the Buehler method (20 in treatment group); induction concentrations were below those causing irritation, and the induction was made on the backs and not on the flanks of the animals.
GLP</td>
<td>Three groups of ten Male Hartley albino Guinea pigs were used</td>
<td>The test material was AGR 233011, lot TB 860825-5, containing 52.63% cis isomer and 44.91% trans isomer 1,3-dichloropropene. Mineral oil and a solution of DER 331 epoxy resin in Dowanol DPM (dipropylene glycol monomethyl ether) were used as vehicle and positive control respectively</td>
<td>Induction - 0.4 ml of 0,1% of Telone II Soil Fumigant in mineral oil was applied in a Hill Top chamber to the shaved left side of ten animals one a week for a total of 3 consecutive weeks. The chambers were removed after a 6 hours.
A positive control group received two applications of 10% DER 331 epoxy resin in Dowanol DPM. The concentration of DER 331 was decreased to 5% for the third induction and challenge application. A third group of pigs served as vehicle controls and received 3 applications of mineral oil throughout the induction phase.</td>
<td>None of the control Guinea pigs challenged with mineral oil revealed signs of sensitisation. Five of ten animals challenged with 5% DER 331 revealed slight erythema. Nine of ten guinea pigs challenged with 0,1% Telone II revealed slight to moderate erythema.
Potential skin sensitisation in Hartley Albino Guinea Pig after the challenge application
<table>
<tr><th rowspan="2">Group</th><th colspan="2">Incidence of significant responses</th></tr>
<tr><th>24 hours</th><th>48 hours</th></tr>
<tr><td>Control (mineral oil)</td><td>0/10</td><td>0/10</td></tr>
<tr><td>Positive control (DER 331)</td><td>5/10</td><td>4/10</td></tr>
<tr><td>Test</td><td>5/10</td><td>9/10</td></tr>
</table>
</td>
<td>Anonymous 16 (1987)
DAR, Spain, 2018</td>
</tr>
</table>

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
OECD 406, Buehler test Deviations: the number of animals used (12 in treatment group) was lower than that recommended for the Buehler method (20 in treatment group); GLP	Two groups of twelve Dunkin-Hartley female Guinea pigs were used for the main study and in the sighting study, groups of two or more Guinea pigs were used and up to four dose levels were tested on each group of animals	Cis 1,3-dichloropropene (Batch No. 87/RM/716). Arachis oil B.P. was used as a vehicle	Topical inductions – days 1 -3 – 2% (v/v) in arachis oil B.P. Topical inductions – days 4 – 9 – 1% (v/v) in arachis oil B.P. and topical challenge – day 28 – 0.5% (v/v) in arachis oil B.P.	None of the control animals exhibited any signs of sensitization while following the challenge application four test animals responded with slight to moderate erythema. Group / Incidence of significant responses: 24 hours / 48 hours Control (vehicle): 0/12 / 0/12 Test: 4/12 / 2/12	Anonymous 22 (1988)
EEC Method B6, GPMT No deviations GLP	Range-finding test - Two male and two female Dunkin-Hartley Guinea-pigs Main test - ten male and ten female Dunkin-Hartley Guinea-pigs together with a control group of five males and five females	Trans-1,3-Dichloropropene Batch No. TR88001 Purity 96.7%	Intradermal induction: 0.05% (m/v) in corn oil and/or Freunds Complete Adjuvant Topical induction: 10% (m/v) in corn-oil Topical challenge: 5% (m/v) in corn oil	16/20 test animals showed positive responses 24 hours after the removal of the challenge patches and 15/20 of the test animals showed positive responses after 48 hours.	Trans-1,3-Dichloropropene: Anonymous 18 (1988)
OECD 406, GPMT. No deviations GLP	Range-finding test - Two male and two female Dunkin-Hartley Guinea-pigs Main test - ten male and ten female Dunkin-Hartley Guinea-pigs together with a control group of five males and five females	Cis-1-3-Dichloropropene Batch No. ST88/253 Purity 96.9%	Intradermal induction: 0.1% (m/v) in corn oil and/or Freund's Complete Adjuvant Topical induction: 5% (m/v) in corn-oil Topical challenge: 2.5% (m/v) in corn oil	In the Guinea-pig maximisation test of Magnusson and Kligman, all of the twenty test animals showed positive responses at 24 and 1820 at 48 hours after removal of the challenge patches	Anonymous 23 (1989)

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

In a skin sensitization study (Anonymous 16, 1987) conducted according OECD 406 (Buehler test method), 3 groups of 10 male Hartley guinea pigs were exposed to AGR 233011, lot TB 860825-5, containing 52.63% cis isomer and 44.91% trans isomer 1,3-dichloropropene. Mineral oil and a solution of DER 331 epoxy resin in Dowanol DPM (dipropylene glycol monomethyl ether) were used as vehicle and positive control respectively.

Since 0.1% (v/v) TELONE II in mineral oil was not irritating to the skin in a previous study performed in Guinea pig concentration of 0.1% has been used for each induction and challenge exposure.

For the induction phase, an aliquot of 0.4 ml of 0,1% of AGR 233011 in mineral oil was applied in a Hill Top chamber to the shaved left side of ten animals one a week for a total of 3 consecutive weeks. The chambers were removed after a 6 hours exposure period and observations for erythema and edema were recorded the following day. A positive control group of 10 animals received two applications of 10% DER 331 epoxy resin in Dowanol DPM. Due to slight erythema observed in the positive control group after the second induction application, the concentration of DER 331 was decreased to 5% for the third induction and challenge application. A third group of pigs served as vehicle controls and received 3 applications of mineral oil throughout the induction phase.

For the challenge phase, all groups were challenged two weeks with 0.1%, after the last induction application. Test material, positive control and vehicle were applied on the shaved right side of the animals in the same manner as throughout in the induction phase. Application sites were observed and graded for sensitisation response 24 and 48 hours after the challenge application.

None of the Guinea pigs challenged with mineral oil revealed signs of sensitisation. Five of ten animals challenged with 5% DER 331 revealed slight erythema. Nine of ten guinea pigs challenged with 0,1% AGR 233011 revealed slight to moderate erythema.

In the second skin sensitization study (Anonymous 22, 1988), the resolved cis-isomer of 1,3-dichloropropene (1,3-D-cis) was tested for skin sensitisation in 12 Guinea pigs. A modified Buehler test was performed using 9 induction applications in a 2-week period. For the first 3 inductions 2% 1,3-D-cis in arachis oil was used which was reduced to 1% for the remainder of the induction period. After a 2-week recovery period the challenge application was made using 0.5% 1,3-D-cis and a control group, treated with vehicle only during the induction period, was similarly challenged. Following the challenge application 4 test animals responded. It was concluded that the cis-isomer of 1,3-dichloropropene showed a potential to produce skin sensitisation in this test.

In the third study (Anonymous 18, 1988), the skin sensitisation potential of the test material (trans-1,3-dichloropropene) was assessed using the maximisation method of Magnusson and Kligman (1969).

Range finding tests - The purpose of the range finding test was to determine the concentrations of test material to be used for intradermal induction, topical induction and topical challenge in the main study. Two male and two female Dunkin-Hartley Guinea pigs were closely shorn in the shoulder region using electric clippers followed by an electric razor. 0.1 ml doses of several dilutions of the test material were injected intradermally on each side of the mid-line. The animals were examined on the following day to determine the maximum concentration that could be used in the main test without causing untoward toxicity. The flank of each animal in further groups of two male and two female guinea pigs, was closely shorn. 0,3 ml doses of several dilutions of the test material were absorbed onto 16 cm^2 Whatman No. 3 filter paper patches. The patches were applied to skin on the shorn flanks, covered by occlusive tape, and retained by an elastic adhesive bandage for 24 hours. After removal of the patches and bandages the dermal test sites were examined for signs of irritation which were scored using a four-point scale. The concentration selected for topical induction in the main test was that which just caused irritation and the concentration chosen for topical challenge was that which was just non-irritant.

Main test-

The main test was conducted using a group of ten male and ten female Dunkin-Hartley guinea pigs together with a control group of five males and five females. Individual bodyweights were recorded at the beginning and at the end of the main study. The test procedure was divided into two stages:

Induction - The animals were closely shorn in the shoulder region using electric clippers followed by an electric razor; two rows of intradermal injections were made, one on either side of the mid-line, as follows:

Test animals

Anterior sites 0.1 ml of Freunds Complete Adjuvant (FCA)

Middle sites 0.1 ml of test material in vehicle Posterior sites 0.1 ml of test material in 50:50 FCA/vehicle

Control animals

Anterior sites 0.1 ml of FCA Middle sites 0.1 ml of vehicle

Posterior sites 0.1 ml of 50:50 FCA/vehicle

One week after induction by intradermal injection, the same area of dorsal skin was shaven using electric clippers only. A 16 cm^2 patch of Whatman No. 3 filter paper was moistened with 0.3 ml of the appropriately diluted test material and placed over the sites of intradermal injections. The patches were covered with occlusive tape and held in place by elastic adhesive bandage for 48 hours. Similar patches of filter paper moistened with the vehicle alone were applied to the control group guinea pigs. Any abnormal reactions to the induction procedure were recorded.

Challenge phase was carried out three weeks after the intradermal phase of induction. Hair was removed from one flank of all test and control animals by clipping and shaving. A 4 cm^2 patch of Whatman No. 3 filter paper, moistened with 0.1 ml of the appropriate dilution of test material, was placed on the shaven area, covered by occlusive tape and held in position by elastic adhesive bandage. Control group animals were treated with the same formulation of test material that was applied to test group animals. After 24 hours the patches and bandages were removed, and the challenge sites examined for any response. The response was scored using a four-point scale.

The result of the test is expressed as the number of positive responses shown by the test animals at 24 and/or 48 hours after removal of the challenge patches.

Table 28: Skin sensitization results following occluded topical challenge with Trans-1,3-Dicloropropene in guinea pigs (Magnusson and Kligman Test)

Group/Number of animals tested	Response to challenge		
	Time after challenge		
	0-hour	24-hour	48-hour
Test group/20	16/20	16/20	15/20
Control group/10	0/10	0/10	0/10

Range finding tests were conducted to determine the concentration of the test material to be used for intradermal induction, topical induction and topical challenge in the main test.

Based on the range finding tests, the following concentrations of test material were selected for the main study:

Intradermal induction: 0.05% (m/v) in corn oil and/or Freunds Complete Adjuvant

Topical induction: 10% (m/v) in corn-oil

Topical challenge: 5% (m/v) in corn oil

Sixteen of the twenty test animals showed positive responses 24 hours after the removal of the challenge patches and fifteen of the test animals showed positive responses after 48 hours.

In the last OECD guideline 406 (GLP) study (Anonymous 23, 1989), the skin sensitisation potential of the test material (cis-1,3-dichloropropene) was assessed using the maximisation method of Magnusson and Kligman (1969).

Range finding tests - The purpose of the range finding test was to determine the concentrations of test material to be used for intradermal induction, topical induction and topical challenge in the main study. Two male and two female Dunkin-Hartley guinea pigs were closely shorn in the shoulder region using electric clippers followed by an electric razor. 0.1 ml doses of several dilutions of the test material were injected intradermally on each side of the mid-line. The animals were examined on the following day to determine the maximum concentration that could be used in the main test without causing untoward toxicity. The flank of each animal in further groups of two male and two female guinea pigs, was closely shorn. 0,3 ml doses of several dilutions of the test material were absorbed onto 16 cm^2 Whatman No. 3 filter paper patches. The patches were applied to skin on the shorn flanks, covered by occlusive tape, and retained by an elastic adhesive bandage for 24 hours. After removal of the patches and bandages the dermal test sites were examined for signs of irritation which were scored using a four-point scale. The concentration selected for topical induction in the main test was that which just caused irritation and the concentration chosen for topical challenge was that which was just non-irritant.

Main test-

The main test was conducted using a group of ten male and ten female Dunkin-Hartley guinea pigs together with a control group of five males and five females. Individual bodyweights were recorded at the beginning and at the end of the main study. The test procedure was divided into two stages:

Induction - The animals were closely shorn in the shoulder region using electric clippers followed by an electric razor; two rows of intradermal injections were made, one on either side of the mid-line, as follows:

Test animals

Anterior sites 0.1 ml of Freund’s Complete Adjuvant (FCA)

Middle sites 0.1 ml of test material in vehicle

Posterior sites 0.1 ml of test material in 50:50 FCA/vehicle

Control animals

Anterior sites 0.1 ml of FCA

Middle sites 0.1 ml of vehicle

Posterior sites 0.1 ml of 50:50 FCA/vehicle

One week after induction by intradermal injection, the same area of dorsal skin was shaven using electric clippers only. A 16 cm^2 patch of Whatman No. 3 filter paper was moistened with 0.3 ml of the appropriately diluted test material and placed over the sites of intradermal injections. The patches were covered with occlusive tape and held in place by elastic adhesive bandage for 48 hours. Similar patches of filter paper moistened with the vehicle alone were applied to the control group guinea pigs. Any abnormal reactions to the induction procedure were recorded.

Challenge phase was carried out three weeks after the intradermal phase of induction. Hair was removed from one flank of all test and control animals by clipping and shaving. A 4 cm^2 patch of Whatman No. 3 filter paper, moistened with 0.1 ml of the appropriate dilution of test material, was placed on the shaven area, covered by occlusive tape and held in position by elastic adhesive bandage. Control group animals were treated with the same formulation of test material that was applied to test group animals. After 24 hours the patches and bandages were removed, and the challenge sites examined for any response. The response was scored using a four-point scale.

The result of the test is expressed as the number of positive responses shown by the test animals at 24 and/or 48 hours after removal of the challenge patches.

Table 29: Skin sensitization results following occluded topical challenge with Cis-1,3-Dicloropropene in guinea pigs (Magnusson and Kligman Test)

Group/Number of animals tested	Response to challenge		
	Time after challenge		
	0-hour	24-hour	48-hour
Test group/20	20/20	20/20	18/20
Control group/10	0/10	0/10	0/10

Range finding tests were conducted to determine the concentration of the test material to be used for intradermal induction, topical induction and topical challenge in the main test.

Based on the range finding tests, the following concentrations of test material were selected for the main study:

Intradermal induction: 0.1% (m/v) in corn oil and/or Freund's Complete Adjuvant

Topical induction: 5% (m/v) in corn-oil

Topical challenge: 2.5% (m/v) in corn oil

All of the twenty test animals showed positive responses 24 hours after the removal of the challenge patches and eighteen of the test animals showed positive responses after 48 hours.

In the guinea-pig maximisation test of Magnusson and Kligman, all of the twenty test animals showed positive responses at 24 and/or 48 hours after removal of the challenge patches.

10.7.2 Comparison with the CLP criteria

The skin sensitization potential of 1,3-D (mix of isomers) was investigated in ten guinea pigs in a Buehler test conducted according to OECD TG 406 in which the animals were induced with the substance at 0.1 % v/v once weekly for 3 weeks and challenged at the same concentration two weeks after the last induction (Anonymous 16, 1987). Slight to moderate erythema was observed in 9/10 Guinea pigs (90%) challenged with 0.1% v/v 1,3-D. The CLP criteria for classification in Skin Sensitisation Category 1A are met since > 15% of animals tested in the Buehler assay responded at a topical induction dose ≤ 0.2%. Hence on the basis of these findings,1,3-D (mix of isomers) meets the CLP criteria for classification in Skin Sensitisation Category 1A (H317) *"May cause an allergic reaction."*

The skin sensitization potential of cis 1,3-D was investigated in twelve guinea pigs in a modified Buehler test conducted according to OECD TG 406 in which the animals received 9 induction applications during a 2-week period (Anonymous 22, 1988). The first 3 inductions used 2% cis 1,3-D, whereas the remaining inductions used the substance at 1%. Two weeks after the last inductions, animals were challenged with 0.5% cis 1,3-D. On challenge, 4/12 (33.3%) animals responded with slight to moderate erythema indicating that cis 1,3-D has skin sensitization potential. The CLP criteria for classification in Skin Sensitisation Category 1B are met since >15% of animals tested in the Buehler assay responded at a topical induction dose ≥ 0.2% and ≤ 20 %. Hence, on the basis of these findings, cis 1,3-D meets the CLP criteria for classification in Skin sensitisation Category 1B (H317) *"May cause an allergic reaction."*, however topical induction dose ≤ 0.2% of cis-1,3-D was not tested to exclude sensitisation response in ≥15% animals and subcategory 1A for skin sensitisation, consequently.

In the further study, the skin sensitization potential of cis 1,3-D was investigated in a Guinea pig maximization test (GPMT; according to Magnusson and Kligman) in accordance with OECD TG 406 (Anonymous 23, 1989). In the study 20 animals received an induction dose at 0.1% m/v intradermally, a topical induction dose at 5% m/v and a topical challenge at 2.5% m/v. Positive responses were observed in 20/20 (100%) of the animals 24 hours after topical challenge and in 18/20 (90%) of the animals after challenge. The CLP criteria for classification in Skin Sensitisation Category 1A are met since ≥ 30% of animals tested in the GPMT responded at an intradermal induction dose ≤ 0.1%. Hence, on the basis of these findings, cis 1,3-D meets the CLP criteria for classification in Skin Sensitisation Category 1A (H317) *"May cause an allergic reaction."*

The skin sensitization potential of trans 1,3-D was investigated in Guinea pigs in a study conducted according to EEC Method B6 (Guinea pigs maximization test (GPMT) according to Magnusson and Klingman) in which 20 animals received an induction dose at 0.05% m/v intradermally, a topical induction dose at 10% m/v and a topical challenge at 5% m/v (Anonymous 18 (1988)). Positive responses were observed in 16/20 (80%) of the animals 24 hours after topical challenge and in 15/20 (75%) of the animals 48 hours after challenge. The CLP criteria for classification in Skin Sensitisation Category 1A are met since ≥ 30% of animals tested in the GPMT responded at an intradermal induction dose ≤ 0.1%. Hence, on the basis of these findings, trans 1,3-D meets the CLP criteria for classification in Skin Sensitisation Category 1A (H317) *"May cause an allergic reaction."*

In conclusion, the evaluation of the available experimental data on 1,3-D (mix of isomers), cis 1,3-D and trans 1,3-D indicates that these substances have a comparable profile with respect to the potential for skin sensitisation. These substances respectively meet the CLP criteria for classification in Skin Sensitisation Category 1A (H317) *"May cause an allergic reaction."*

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the data presented, 1,3-D (mix of isomers), (Z)-1,3-D (cis-isomers) and (E)-1,3-D (trans- somers) should be classified in accordance with the CLP Regulation as:

Skin Sensitisation Category 1A, H317, May cause an allergic reaction.

10.8 Germ cell mutagenicity

Table 30: Summary table of mutagenicity/genotoxicity tests in vitro

Method	Results	Remarks	Reference
In vitro			
1) Bacterial Gene mutation a) Plate incorporation method ±S9 (*E. coli* B/r WP2, *S. typhimurium* G46, TA1535, TA1537, TA1538, TA100, TA98) b) Host mediated assay (*S. typhimurium* G46; host ICR male mice) c) Rec-assay (*B. subtilis* H17 Rec^+, M45 Rec^-) **1,3-D** 96.1% purity (49.8% cis, 46.3% trans) Batch: EC-440F This study is pre-guideline GLP-No	a) In TA1535 and TA100 was **positive** (>2-fold increase in revertants), and in TA98 and G46, weak positive (±S9, 1.5-2-fold increase in revertants). b) Negative c) Positive	**Not acceptable** Due to the poor characterization on test substance used and significant **OECD Guideline deviations** in its conduct. **Solvent DMSO**.	Anonymous 41 (1978)
2) Bacterial Gene mutation Plate incorporation method ±S9 (*S. typhimurium* TA1535, TA1538, TA100, TA98). **1,3-D** 95% purity (51.3% cis, 43.7% trans) + **epichlorhydrin** (0.6%) Unknown batch. **Cis-1,3-D** >99% purity Batch: 2700 (Also tested in TA 100 (±S9 ± glutathione) This study is pre-guideline GLP-No	**1,3-D Positive** in TA1535, TA100 (±S9). **Cis-1,3-D Positive** in TA1535, TA100 and TA98 (±S9) Mutagenicity decreased in the presence of glutathione.	**Not acceptable.** The batch used in this study does not comply with specification of the current batches, as it **contains epichlorohydrin**, in addition, the study has important OECD Guideline deviations in its conduct. **Solvent Not stated**	Anonymous 28 (1978)
3) Bacterial Gene mutation Pre-incubation method (*S. typhimurium* TA100) S9 from lungs of uninduced control or 1,3-D inhalation exposed mice (± glutathione).	**Negative**	**Not acceptable**	Anonymous 32 (1996)

Method	Results	Remarks	Reference
1,3-D 97.6% purity (51.7% cis, 45.9% trans) Batch: AGR295646. **Without epoxidized soybean oil (ESO) stabilizer** (purified by passing through a silicic acid column). OECD Guideline 471 GLP-Yes		The batch used in this study does not comply with specification of the current batches. Only one strain was used in this assay. Since other studies provide the same end-point of genotoxicity, the study is considered as not acceptable**.** Significant deviations from OECD test guideline 471. **Solvent: ethanol**	
4) Bacterial Gene mutation Preincubation method ±S9 (*S. typhimurium* TA1535, TA100) **1,3-D** 97.6% purity (49.9% cis, 47.7% trans) **Without stabilizer and purified by passing through a silicic acid column** Batch: TSN100704A. **1,3-D** 96.6% purity (49.3% cis, 47.3% trans) **Without stabilizer and purified by passing through a silicic acid column + epichlorhydrin** (1.46%) Batch: TSN100704B	**1,3-D** – Negative **1,3-D + epichlorhydrin Positive** in TA1535 (-S9)	**Not acceptable** The current batches does not contain epichlorhydrin as stabilizer, thus the study results does not provided useful information for the current evaluation of test substance. Significant deviations from OECD test guideline 471. **Only two strains were used** instead of five recommended. **Solvent: ethanol**	Anonymous 34 (1997)
5) Bacterial Gene mutation preincubation method ±S9 (*S. typhimurium* TA1537, TA1535, TA98, TA100; *E. coli* WP2 *uvr*A) **1,3-D** 96% purity (49.3% cis,46.7% trans). Batch: TSN101035 OECD Guideline 472 GLP-Yes	**Positive** TA1535 and TA100 without S9. TA 1535 with S9 (Mutagenicity decreased in the presence of S9)	**Acceptable.** **Solvent: ethanol**	Anonymous 35 (1999)
6) Bacterial Gene mutation Preincubation method (*S. typhimurium* TA1535, TA100) in the presence and absence of purified rat liver microsomes **1,3-D** 99.64% purity (51.36% cis,48.28% trans). Batch: TSN100704 unstabilised Guideline-Not stated GLP-Yes	**Positive** for TA1535 and TA100 strains (presence and absence of purified rat liver microsomes)	**Supplemental**. **Only 2 strains.** The results confirm the positive result of Anonymous 35 (1999). ESO was not tested in the batch. **(DMSO used as dosing solvent)**	Anonymous 33 (2009)
7) Bacterial Gene mutation Evaluation of species differences in the *in vitro* activation of 1,3-dichloropropene in the *Salmonella* reverse mutation assay. Preincubation method (*S. typhimurium* TA1535) four activation conditions: 1) without exogenous metabolic activation, 2) with purified microsomes (M), 3) with S100 (cytosol) and glutathione (GSH), and 4) with M, S100, and GSH. S100 and M from each of the following species was used: 1) rat, 2) mouse, and 3) human. **1,3-D** batch TSN100704 (uninhibited) same batch as study 6 = 99.64% purity Guideline-None GLP-Yes	**Positive** for TA1535 (-M) **For all 3 species:** **Positive** for TA1535 (+M) **Negative** for TA1535 (+S100, +GSH) **Negative** for TA1535 (+M, +S100, +GSH)	**Supplemental**. **Only a single tester strain** was evaluated (TA1535). **Solvent: ethanol**	Anonymous 31 (2004)

Method	Results	Remarks	Reference
8) Bacterial Gene mutation Preincubation method ±S9 (*S. typhimurium*, TA100, TA98) **1,3-D** purity not given (49.4% cis,42.5% trans). Lot: TB831213-4 – dosed to mice to provide test urine. **Metabolites:** • N-acetyl-S-(3-chloroprop-2-enyl) cysteine (**N-acetylcysteine conjugate**) (Mouse & Rat metabolite) • 3-chloroprop-2-enyl-S-cysteine (**cysteine conjugate**) (Putative metabolite) • N-acetyl-S-(3-chloroprop-2-enyl) cysteine sulfoxide/sulfone (**sulfoxide/sulfone conjugate**) (Mouse and Rat metabolite) • S-(3-chloro-2-propenyl) mercaptoacetic acid (**thioglycolic acid conjugate**) (Mouse metabolite) • bis-(3-chloro-2-propenyl) disulfide (**disulfide**) (Mouse metabolite) (tested -S9 only) OECD 471 GLP-Yes	**Mouse Urine -** Negative for TA 100 and TA98 ± S9. **N-acetylcysteine conjugate** Positive for TA100 (-S9) (single experiment) **Cysteine conjugate -** Positive for TA100 (-S9) & TA98 (-S9). Negative in the presence of a kidney homogenate. Poor substrate of hepatic and renal beta-lyase *in vitro.* **sulfoxide/sulfone conjugate -** Positive for TA100 (-S9) & TA98 (-S9) **thioglycolic acid conjugate** - Positive for TA100 (-S9) **Disulfide –** Negative (-S9) (single experiment)	**Supplemental** Mainly due to deviations form OECD guideline 471: Purity of the test compounds (metabolites) not given. **Only two strains used. No individual plate counts. No second independent experiment with some test compounds. No assays in the presence of S9 with some test compounds.**	Anonymous 39 (1989)
9) Bacterial Gene Mutation Cis-1,3-dichloropropene (purity/Lot not stated) Tested in TA100, TA1535 strains only, no information on vehicle used Guideline not stated GLP - No	In this study, the addition of cis-1,3-dichloropropene to cultures of Salmonella TA100 and TA1535 resulted in dose-dependent increases in the frequencies of reversion. Both of these strains are sensitive to the induction of base-pair substitutions and the results obtained in the most sensitive strain TA100 highlighted the importance of the glutathione transferases in affording protection against potentially mutagenic electrophiles	**Supplemental** Mainly due to deviations form OECD guideline 471: Purity of the test compound not given. **Only two strains used. No individual plate counts. No second independent experiment with some test compound**	Anonymous 42 (1978)
10) Bacterial Gene Mutation Cis-1,3-Dichloropropene (Batch 27000, purity > 99%), solutions prepared in dimethylsulphoxide Tested in TA 98, TA100, TA1535 and TA 1538 strains Guidelines – not stated GLP - No	Cis-1,3-Dichloroprene induced a dose-related increase in the reverse-mutation rate of Salmonella typhimurium TA1535, TA98 and TA100 both in the presence and absence of a rat liver microsomal activation system. The incorporation of 5 mM glutathione afforded protection against the	**Supplemental** Mainly due to deviations form OECD guideline 471: **Only four strains used. No individual plate counts. No second independent experiment with some test compound.**	Anonymous 43 (1978)

Method	Results	Remarks	Reference
	mutagenicity of cis-1,3-dichloropropene up to a concentration of 250 µg per plate both in the presence and absence of a rat liver microsomal activation system.		
11) Bacterial Gene Mutation Equivalent or similar to OECD 471 Cis-1,3-Dichloropropene (Batch Number 88009/1986, purity – 94.51% - 97.51%), testing concentrations were prepared as solutions in DMSO GLP - Yes	The addition of cis-1,3-dichloropropene to cultures of Escherichia coli WP2 uvrA pkm101, Salmonella typhimurium TA 1535 and TA 100 showed reproducible, dose-related increases in reverse gene mutation in the presence and in the absence of rat liver S9 fraction. Smaller dose-related increases were also observed in S, typhimurium TA98 both in the presence and in the absence of rat liver S9 fraction. No increases were seen in S. typhimurium TA1537 or TA1538. The substance is considered to be potentially mutagenic under the conditions of the test.	A GLP -compliant study was conducted to a test method similar to OECD Guideline 471.The mutagenic activity of cis-1,3 - dichloropropene was investigated in agar layer cultures of selected bacterial tester strains of Salmonella typhimurium and Escherichia coli. Assays were performed in both the presence and absence of S9 microsomal fraction obtained from a liver homogenate of rat pre-treated with Aroclor 1254. It was concluded that cis-1,3 - dichloropropene was direct-acting bacterial mutagen under the experimental conditions employed. A mutagenic dose of the test material was tested in the presence of a range of concentrations of glutathione. Glutathione reduced the mutagenicity of the test material in the presence and absence of S9 fraction.	Anonymous 44 (1990)
***In vitro* mammalian genotoxicity studies**			
12) *In vitro* mammalian cell Gene mutation assay HPRT test. Chinese hamster ovary cells (CHO-K_1-BH_4) ±S9. **1,3-D** 92.1% purity (48.9% cis, 43.2% trans) Batch: TB831213-4 OECD Guideline 476 GLP-Yes	**Negative** - 1,3-dichloropropene did not induce gene mutations at the HGPRT-locus in CHO cells, under the conditions of this study.	**Supplemental**. Batch used does not comply with the current specifications. **(Solvent DMSO)**	Anonymous 36 (1986)
13) Chromosome Aberration assay. Chinese hamster lung (CHL) cells ±S9. **1,3-D** 95.3% purity Batch: 90.10 APR/88 HFK880423-0 OECD Guideline 473 GLP-Yes	**Positive** for: structural aberrations (±S9) in all treatments; numerical aberrations (-S9) only after 48 h treatment	**Supplementary.** Batch analysis is not available, only the purity is known. Cytotoxicity measure used is recommended for cell lines in OECD 473 and several concentrations scored for aberrations exceed the acceptable toxicity limit. pH and osmolarity changes were not reported. Insufficient metaphases scored. **(Solvent DMSO)**	Anonymous 38 (1988)

Method	Results	Remarks	Reference
14) Sister-chromatid exchange (SCE) test with human lymphocytes in vitro **1,3-D** 95% Purity (cis/trans mixture 81:1**)** OECD Guideline 479 GLP-No	**Positive** -increases in SCE-frequencies in human lymphocytes (with and also without addition of an exogenous metabolizing system)	**Not acceptable** OECD guideline 479 SCE test was deleted on 2nd April 2014 – DMSO solvent Samples of bone marrow should be taken at least twice (from independent groups of animals), starting not earlier than 24 hours after treatment, but not extending beyond 48 hours after treatment with appropriate interval(s) between samples. At least 4000 immature erythrocytes per animal should have been scored for the incidence of micronucleated immature erythrocytes Limited information on batch available, and not representative of technical material	Kevekordes *et al.* (1996)
15) UDS assay Primary hepatocytes from male CD Fisher 344 rats. **1,3-D** 92.1% purity (49.5% cis, 42.6% trans) Batch: TB831213-4 OECD Guideline 482 GLP-Yes	**Negative** - 1,3-dichloropropene did not induce DNA damage leading to increased repair synthesis in treated rat hepatocytes, under the conditions of this study.	**Supplemental**. OECD 482 was deleted in 2014 and the endpoint is no longer a data requirement. Only 30 cells per concentration (instead of 50 recommended by ECD 482) scored for UDS	Anonymous 37 (1985)
16) *In vitro* DNA binding (adduct) assay (Calf thymus DNA) in the presence or absence of potentially activating enzymes (S9) and physiological levels of GSH. **^{14}C-1,3-D** >99% purity. Batch: B463-48; expected cis/trans ratio to be approximately 50/50 since is analytical grade. Guidelines: There is no available OECD Guideline. GLP: No	**Negative -** 1,3-dichloropropene did not bind calf thymus DNA in vitro, either in the absence or presence of potential activating enzymes, under the conditions of this study.	**Supplemental**. Assay not described by OECD guideline	Anonymous 40 (1997)
17) *In vitro* mammalian chromosome aberration in Chinese hamster ovary cells Chinese hamster ovary cells (CHO-K_1) ±S9 Cis-1,3-Dichloroproene 94.51% - 97.51% purity, Batch: 8800/1986 EPA Guideline 84-2 GLP-Yes	The test material induced chromosome damage in the presence but not the absence of metabolic activation by S9 mix in cultured CHO cells. When physiological strength (5mM) glutathione was added to test material treated cultures in the presence of S9 mix, the extent of chromosome damage was reduced to levels observed in the vehicle control cultures, under the standard test experimental conditions	**Supplemental – GLP study conducted in accordance with appropriate test guidelines with no or minor deviations**	Anonymous 45 (1991)

Method	Results	Remarks	Reference
	as described in the guidelines.		
18) Genotoxicity Studies with Trans-1,3-Dichloropropene: In-vitro chromosome studies with Trans-1,3-Dichloropropene Trans-1,3-Dichlorpropene, Batch No. TR8801, Purity - 96.7% EPA Pesticide Assessment Guideline 84-2	Under the experimental conditions, it was concluded that trans-1,3-Dichloropropene induced chromosome damage in cultured CHO cells in the presence of a rat liver metabolic activation system (S9) and no clastogenic activity was observed in the absence of the S9 metabolic activation system	**Supplemental – GLP study conducted in accordance with appropriate test guidelines with no or minor deviations**	Anonymous 46 (1989)

Table 31: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method	Results	Remarks	Reference
In vivo genotoxicity studies with somatic mammalian cells			
1) Gene Mutation Assay at the *cII* Locus in Male Big Blue Transgenic F344 Rats **1,3-D**, 33.7% Purity (a mixture of the cis and trans Isomers) microencapsulated in 80% starch and 20% sucrose. Lot number M04032018NP OECD Guideline 488 GLP-Yes	**Negative** 1,3-dichloropropene did not induce mutations at the *cII* gene in the liver and kidneys of male Big Blue rats.	**Acceptable** GLP study follows OECD TG 488, 2013**.**	Anonymous 24 (2018)
2) Inhalation - Gene mutation assay at the *lacI* target gene of transgenic Big Blue B6C3F1 male mice **1,3-D** 96% purity (49.3% cis, 46.7% trans) Batch: TSN101035. Study was similar to OECD Guideline 488 GLP-Yes	**Negative** 1,3-dichloropropene did not induce *lacI* mutations either in the liver or the lung tissue of transgenic Big Blue B6C3F1 male mice	**Acceptable** *with reservations.* The exposure to test substance was only 2 weeks (6 h/day, 5 days/week) instead of the daily treatment for a period of 28 days recommended in the current OECD Guideline 488 (July 2013), to produce a sufficient accumulation of mutation and detect mutation in slowly proliferating organs. However, the study does have a 17-day post-exposure expression period.	Anonymous 25 (1997)

Method	Results	Remarks	Reference
3) Oral gavage - Micronucleus assay - Bone marrow cells from male and female CD-1 (ICR) BR mice. **1,3-D** 92.1% purity (49.5% cis, 42.6% trans) Batch: TB831213-4 OECD Guideline 474 GLP-Yes	**Negative** At 380 mg/kg (high dose), 1 male in the 24 h group and 3 males in the 48-h group did not survive, and there was toxicity for the bone marrow in females of the 48-h group.	**Supplementary** The ratio of polychromatic to normochromatic erythrocytes was determined for each animal by counting approximately 200 erythrocytes instead of the 500 recommended. Only 1000 immature erythrocyte per animal were scored instead of 4000 immature erythrocytes recommended in the Guideline OECD 474 (2016). Reporting detail is limited, neither the individual MNPCE frequencies per animal nor HCD are reported	Anonymous 26 (1985)
4) Intraperitoneal - Micronucleus assay - Bone marrow cells from male B6C3F1 mice. **1,3-D** Purity not given. Unknown batch, purity or original source OECD Guideline 474 GLP-Yes	**Equivocal** But only when tested at 150 mg/kg or greater after one exposure and 48 h harvest. The increases were statistically significant when compared to control but within reported HCD **Negative** for all other conditions. 2 tests where mice were administered 1,3-D for 3 consecutive days at 31, 62.5 and 125 mg/kg were negative.	**Supplementary** Insufficient characterization information for the chemical. The I.P. route of administration is not recommended. Only 200 erythrocytes counted for ratio of polychromatic (PCE) to normochromatic erythrocytes (500 recommended) and only 2000 PCE scored for micronuclei (4000 recommended).	Shelby *et al.* (1993)
5) Genotoxic activity of 1,3-dichloropropene in a battery of *in vivo* short-term tests in male Sprague-Dawley rats. Anticipated cis/trans ratio of approximately 50/50 since material is analytical grade. **a) Micronucleus tests** in rat liver, bone marrow and spleen cells (DMSO as vehicle). OECD Guideline 474 GLP-Yes **b) DNA damage, evaluated by the alkaline elution technique**; (DMSO as vehicle).liver, lung, gastric mucosa, kidney, bone marrow and brain. (+influence of GSH depletion and cytochrome P450 activity inhibition) Non guideline GLP-No **c) UDS** in hepatocytes with the *in vivo/in vitro* technique.(DMSO as vehicle) **1,3-D** 98% purity. Batch: 9801604 OECD Guideline TG 486 (1997) GLP-No	**a) Negative –** all three tissues. NDMA (positive control) induced, a clear-cut clastogenic response in the three target organs. **b) Positive** only in gastric mucosa, kidney and liver with a maximum in the first 3 h. Liver is the most sensitive (The effect induced by 1,3-D did not increase with DEM or BSO and decreased with MS) **c) Negative**	**a) Supplementary**. Deviations from OECD 474 : i) animals were partially hepatectomized; ii) only one sample, 48 h post-dosing, for each tissue, instead of at least two recommended; iv) little information about methodology, individual animal data and HCD **b) Supplementary** Non-guideline study noted for its extreme variability and inconsistency. No positive control. Data for MS, DEM and BSO administered alone are not shown in the published paper. **c) Acceptable** *with some reservations.* Deviations from OECD 486 (1997): a) only one dose level of the test substance; b) only males; c) only 1 sampling time used, whereas 2 are recommended (2-4 h and 12-16 h after dosing; d) no information about cell viability; e) no information about toxicity for animals, f) individual slide and animal data not provided; and g) historical control data not given.	Ghia *et al.* (1993)

Method	Results	Remarks	Reference
6) DNA damage in female rat liver - Alkaline elution assay. **1,3-D** purity not reported. Not a guideline study GLP-No	**Positive** 1,3-dichloropropene caused DNA damage in rat liver at 94 mg/kg.	**Unacceptable**. Few details on methodology and doses tested. Purity of the test substance was not reported. Non-guideline study. A total of 49 compounds were tested – only 1,3-D is considered here.	Kitchin, K.T. & Brown, J.L. (1994)
7) In vivo mammalian (NMRI mice) erythrocyte micronucleus test **1,3-D** 95% Purity (cis/trans mixture 81:1**)** OECD Guideline 474 GLP-No	**Positive -** increase in the frequency of micronuclei (MN) in female mice but not in males. Unexplained.	**Unacceptable** Deviations from OECD guideline 474: Bone marrow sampled once at 48 hours, not twice (independent groups), 24 hours to 48 hours after treatment. 1000 PCE/animal were scored for presence of micronuclei, instead of recommended 4000. Only 1 animal of each sex in the solvent control groups	Kevekordes *et al.* (1996)
8) Micronucleus Test with Trans-D in mice Trans-1,3-Dichloropropene (Trans-D) Batch No. A4308 05008, Purity 96.7% OECD Guideline 474 GLP Yes	Negative – Based on the conditions used in the study, it was concluded that Trans-D did not produce micronuclei in polychromatic erythrocytes in mice	**Acceptable**	Anonymous 47 (1999)
9) *In Vivo* Micronucleus Assay of 1,3-Dichloropropene by Oral Gavage in CD-1 Mice **1,3-D** 97.8% purity (53.4% cis, 44.4% trans) Batch: 6B-6D OECD Guideline 474 GLP - Yes	Negative – Based on the conditions used in the study, it was concluded that 1,3-D demonstrated a negative response for induction of peripheral blood micronuclei at all dose levels in mice	**Acceptable**	Anonymous 145 (2023)
In vivo genotoxicity studies with germinal cells			
1) Drosophila melanogaster - sex-linked recessive lethals (SLRL) induction, and reciprocal translocations (RT) **1,3-D** 95.5% purity - Unknown batch. No information on isomer composition is available in the study report. OECD Guideline 477 GLP-No	**SLRL – Weak Positive/ Equivocal** - Positive in only one trial indicating that the positive response was not reproducible. **RT - Negative**	**Not acceptable** Insufficient detail reported. Deviations from OECD 477 (1984: a) no details on methodology; b) the age of insects, and the number of males treated, F2 cultures established and F2 cultures without progeny were not reported; c) statistical significance of the increase in the frequency of SLRL caused by 1,3-dichloropropene not reported.. **OECD 477 (1984) was deleted on 2nd April 2014**	Valencia *et al.* (1985)
2) Dominant lethal assay in CD (Sprague-Dawley derived) rats **1,3-D** 96% purity (49.3% cis, 46.7% trans) Batch: TSN101035 **1,3-D** 96.5% purity (49.87% cis, 46.59% trans) Batch: TSN101299 OECD Guideline 478 GLP-Yes	**Negative**	**Acceptable**	Anonymous 27 (1997)

Table 32: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
None available				

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

In vitro data

Bacterial Mutagenicity
A total of eleven bacterial mutagenicity studies are presented and assessed.

The study by Anonymous 41 (1978), describes a reverse mutation assay in *E. coli* and *S. typhimurium* as well as a rec-assay in *B. subtilis* and a host-mediated assay. The reverse mutation assay was conducted prior to establishment of OECD TG 471 and consequently the study deviates significantly from the requirements of OECD TG 471 (1997). Key deviations include the use of some non-guideline strains of bacteria, +S9 positive controls were not conducted for all strains, unclear reporting regarding number of plates per concentration and a lack of adequate test article characterisation information. As a consequence, the reported data are considered to be of limited reliability. The assay was conducted using the plate incorporation method. One strain, *E. coli B/r WP2* and six *S. typhimurium* strains (G46, TA1535, TA100, TA1537, TA1538 and TA98) were used. S9 was derived from livers of male Wistar rats induced with PCB 500 mg/kg (Kaneklor 400). The negative control was DMSO, the solvent. Appropriate concurrent positive controls with and without S9 were used. 1,3-D was tested at concentrations in the range 25-5000 µg/plate (*E. coli*) or 10-10000 µg/plate in the absence and presence of S9. Concentrations of 250, 500 and/or 1000 µg/plate and above were toxic in strains G46, TA1537, TA98 and TA1538. The test substance was clearly mutagenic (dose-dependent, >3-fold increases in revertants) with and without S9, in strains TA100 and TA1535. Weaker responses were also observed in strains G46 and TA98, with and without S9. The rec-assay was concluded to be qualitative positive, however, the results were compromised by the volatility of 1,3-D, and therefore quantitative assessments were not possible. The host-mediated assay was negative. As both of these assays are not described by OECD guidelines and are no longer a data requirement for genotoxicity assessment, they are considered as supplemental only.

The study by Anonymous 28 (1978) is considered unacceptable because it tested a batch of 1,3-D known to contain epichlorohydrin, added as a stabilizing agent. Epichlorohydrin is an *in vitro* and *in vivo* genotoxicant, and there is no way to determine to what extent the positive responses seen could be attributed to the parent 1,3-D or to the epichlorohydrin contaminant. Therefore, the data from these studies are not considered as acceptable for the assessment of the mutagenicity potential of 1,3-D and are not further discussed.

Two studies by Anonymous 32 (1996) and Anonymous 34 (1997), were designed to evaluate the effects of 1,3-D responses in the bacterial strains shown by Anonymous 28 (1978) to be the most sensitive to 1,3-D. In Anonymous 32 (1996), 1,3-D without epoxidized soybean oil stabilizer (purified by passing through a silicic acid column) was tested to determine if the effects seen by Anonymous 28 (1978) could be attributed to the presence of epichlorohydrin in the technical batch of 1,3-D used. The assay was conducted using *S. typhimurium* TA100 strain in the absence and presence of mouse lung S9. Two types of S9 were used: MLu-C S9, prepared from the lungs of untreated (control) B6C3F1 male mice-C S9) and MLu-T S9) prepared from the lungs of male B6C3F1 mice exposed to 1,3-dichloropropene at 63 ± 7 ppm, 5 days/week for 2.5 weeks by inhalation. Both MLu-C S9 and MLu-T S9 were tested in the presence and absence of glutathione. The assay was performed according to the pre-incubation method. The negative control was ethanol, the solvent. Appropriate positive controls were used with and without S9. The with S9 positive control was specifically validated for use with mouse lung S9.1,3-dichloropropene was tested at 100-450 µg/plate, in the absence of S9, and at 75-1000 µg/plate in the presence of each S9 mix, along with concurrent negative and positive controls. A positive response was considered to be at least a 3-fold increase in the mean number of revertants

per plate compared to the concurrent negative control, that occurred at more than one dose level and was accompanied by a dose response relationship and was demonstrated to be reproducible. There was no precipitation, but cytotoxicity was observed from 225 µg/plate (without S9 mix) and from 300 µg/plate (with S9 mix). 1,3-D was concluded to be negative, under all of the activation conditions tested. Positive controls gave a satisfactory response. Although 1,3-D failed to induce sufficient revertants to reach the criteria for a positive response, a 1.6-fold increase in revertants is apparent in the absence of S9 at a non-toxic concentration (see results table below).

Table 33: Mutagenicity Assay results (Anonymous 32, 1996)

Test article	Dose/ plate (µg)	With S9 mix					Without S9	
		TA100 Revertants/plate				Background Lawn*	Revertants/plate	Background Lawn*
		MLu-C Mean (S.D.)	MLu-C +glu Mean (S.D.)	MLu-T Mean (S.D.)	MLu-T +glu Mean (S.D.)		Without S9 Mean (S.D.)	
Vehicle Control		89 (8)	95 (11)	103 (8)	83 (13)	1	87 (12)	1
1.3-D	75	106 (8)	NT	101 (8)	78 (18)	1	NT	
	100	NT	NT	NT	NT		119 (5)	1
	150	125 (22)	95 (11)	102 (14)	76 (4)	1	141 (4)	1
	225	NT	NT	NT	NT		135 (14)	2
	300	84 (8)	83 (6)	68 (11)	62 (7)	2	133 (8)	2
	450	NT	NT	NT	NT		32 (8)	4
	600	0	0	0	0	5	NT	
	1000	0	0	0	0	5	NT	
Positive Control		1034 (142)	692 (119)	877 (201)	493 (287)	1	717 (70)	1

* Backaround Lawn Code.:
1 – normal, 2 – slightly reduced, 3 – moderately reduced, 4 - extremely reduced, 5 – absent, 6 - obscured by precipitate.
s.p. - .slight precipitate, (requires hand count) mp - moderate precipitate, (required hand count) hp - heavy precipitate
NT – Not tested.

In Anonymous 34 (1997) two batches of 1,3-D, one with 1.5% epichlorohydrin content and one without, were tested in a bacterial mutagenicity assay using pre-incubation methodology, that used just two strains of *S. typhimurium* (TA1535, TA100), with and without a metabolic activation system (S9) from livers of male Sprague-Dawley rats induced with Aroclor 1254. The negative control was ethanol, the solvent. Appropriate positive controls were included. The same criteria for a positive response were used (see Anonymous 32, 1996). In this assay 1,3-D is reported as negative in tester strains TAl00 or TAI535, both in the presence and absence of S9 activation. 1,3-dichlopropene with 1.5% epichlorohydrin caused positive increases (5.4 and 6.0-fold) in tester strain TA1535 in the absence of S9 mix. Based upon these results, it was concluded that 1,3-D *per se* was not genotoxic to the Salmonella tester strains used, and the presence of 1.5% epichlorohydrin, a known *in vitro* and *in vivo* genotoxicant, in the 1,3-D sample elicited a positive response in these strains. Although 1,3-D failed to induce sufficient revertants to reach the criteria for a positive response, dose-related, reproducible increases between 1.5 to 2-fold increases in revertants are apparent under some conditions (see results table below).

Table 34: Mutagenicity Assay results (Anonymous 34, 1997)

Test article	**Dose/ plate (µg)**	**Experiment 1 Mean (S.D)**			**Experiment 2 Mean (S.D)**			**Experiment 3 Mean (S.D)**		
		TA100	**TA1535**	**BL***	**TA100**	**TA1535**	**BL***	**TA100**	**TA1535**	**BL***
With S9										
Vehicle control		131 (15)	13 (4)	1	98 (3)	19 6)	1	84 (14)	16 (2)	1
1,3-D	10	135 (13)	13 (2)	1	95 (9)	14 (3)	1	NC	18 (6)	1
	33.3	137 (16)	19 (2)	1	110 (16)	15 (2)	1	92 (8)	21 (5)	1
	66.7	162 (7)	15 (3)	1	102 (5)	20 (9)	1	112 (3)	18 (7)	1
	100	173 (11)	20 (3)	1	113 (15)	19 (5)	1	109 (17)	28 (1)	1
	333	218 (4)	32 (3)	1	160 (17)	26 (9)	1	172 (14)	48 (6)	1
	667	178 (41)	38 (4)	1	171 (20)	24 (5)	1	111 (15)	37 (5)	2

Test article	Dose/ plate (µg)	Experiment 1 Mean (S.D)			Experiment 2 Mean (S.D)			Experiment 3 Mean (S.D)		
		TA100	TA1535	BL*	TA100	TA1535	BL*	TA100	TA1535	BL*
	1000	93 (82)	49 (11)	2	151 (105)	11 (20)	1/3	0	11 (10)	¾
	2000	0	0	5	0	0	5	0	0	5
Positive control		215 (20)	143 (13)	1	1129 (22)	173 (5)	1	946 (8)	143 (17)	1
Vehicle control		136 (18)	11 (3)	1	96 (8)	13 (6)	1	NT	NT	
1,3-D+1.5% epichlorohydrin	3.33	NT	NT		79 (13)	14 (4)	1	NT	NT	
	6.67	NT	NT		80 (8)	12 (3)	1	NT	NT	
	10	56 (16)	9 (5)	1	92 (12)	11 (4)	1	NT	NT	
	33.3	162 (15)	16 (4)	1	102 (3)	12 (1)	1	NT	NT	
	66.7	172 (8)	22 (2)	1	104 (9)	18 (3)	1	NT	NT	
	100	196 (10)	27 (8)	1	110 (15)	19 (6)	1	NT	NT	
	333	138 (36)	38 (9)	2	144 (6)	33 (6)	1	NT	NT	
	667	0	0	4	181 (16)	22 (24)	1/2	NT	NT	
	1000	0	0	5	NT	NT		NT	NT	
	2000	0	0	4	NT	NT		NT	NT	
Positive control		1068 (104))	153 (15)	1	1107 (86)	151 (12)	1	NT	NT	
Without S9										
Vehicle control		132 (20)	12 (4)	1	79 (4)	14 (3)	1	NT	NT	
1,3-D	6.67	NT	NT		86 (4)	11 (0)	1	NT	NT	
	10	102 (19)	9 (1)	1	63 (5)	16 (1)	1	NT	NT	
	33.3	116 (19)	15 (2)	1	66 (14)	13 (2)	1	NT	NT	
	66.7	133 (37)	13 (1)	1	69 (5)	14 (1)	1	NT	NT	
	100	120 (10)	18 (6)	1	78 (17)	16 (8)	1	NT	NT	
	333	123 (10)	25 (7)	1	58 (4)	7 (4)	3	NT	NT	
	667	56 (5)	9 (8)	3	18 (32)	4 (7)	2/5	NT	NT	
	1000	0	0	5	0	0	5	NT	NT	
	2000	0	0	5	NT	NT		NT	NT	
Positive control		875 (31)	757 (17)	1	946 (32)	778 (53)	1	NT	NT	
Vehicle control		90 (8)	7 (1)	1	67 (5)	15 (4)	1	NT	NT	
1,3-D+1.5% epichlorohydrin	3.33	NT	NT		80 (6)	11 (3)	1	NT	NT	
	6.67	NT	NT		70 (3)	12 (3)	1	NT	NT	
	10	135 (39)	7 (2)	1	70 (4)	13 (4)	1	NT	NT	
	33.3	108 (21)	20 (4)	1	90 (13)	12 (2)	1	NT	NT	
	66.7	124 (14)	32 (10)	1	102 (16)	22 (3)	1	NT	NT	
	100	135 (6)	37 (11)	1	104 (12)	30 (8)	1	NT	NT	
	333	25 (25)	38 (16)	3	130 (3)	53 (27)	1	NT	NT	
	667	0	0	5	90 (46)	90 (19)	1/2	NT	NT	
	1000	0	0	5	NT	NT		NT	NT	
	2000	0	0	5	NT	NT		NT	NT	
Positive control		900 (17))	619 (19	1	845 (33)	722 (38)	1	NT	NT	

* Backaround Lawn Code.:
BL – Background Lawn, 1 – normal, 2 – slightly reduced, 3 – moderately reduced, 4 - extremely reduced, 5 – absent, 6 - obscured by precipitate.
s.p. - .slight precipitate, (requires hand count)
mp - moderate precipitate, (required hand count)
hp - heavy precipitate
NT – Not tested.
NC – No Count

The bacterial mutagenicity study conducted by Anonymous 35 (1999) is fully compliant with OECD TG 471 (1997). The assay was conducted by means of the pre-incubation method, using *S. typhimurium* (TA1535, TA1537, TA98, TA100) and *E. coli* (*WP2 uvrA*) strains, in the presence and absence of S9 activation derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254. The negative control was ethanol, the solvent. Appropriate positive controls were used. The criteria for a positive response were at least 3-fold (TA98, TA1535, TA1537 and WP2 uvrA) or 2-fold (TA100) dose related reproducible increases in the mean revertants per plate of at least one tester strain over the mean revertants per plate of the appropriate negative control.

In the first experiment (Table 35 – Initial test), cytotoxicity was observed from 1000 µg/plate in all strains (with and without S9). There were no revertants either at 3330 µg/plate (all strains) or 1000 µg/plate (TA1537). Positive increases in the mean number of revertants per plate, 6.6-fold (TA1535 without S9), 3-fold (TA1535 with S9) and 2.2-fold (TA100 without S9), were observed at 333 µg/plate (the highest non-toxic concentration). A 1.6-fold increase in TA100 in the presence of S9 was also observed. No increases in the mean number of revertants per plate were observed with any of the remaining tester strain/activation condition combinations. Positive controls gave a satisfactory response.

In the confirmatory mutagenicity assay (Table 36 – Re-test), cytotoxicity, as a reduction in the background lawn, was observed from 1000 µg/plate in all strains (with and without S9). There were no revertants either at 3330 µg/plate (all strains in the presence and absence of S9) or 1000 µg/plate (TA100 in the presence of S9). The concentration of 500 µg/plate caused a reduction in the mean number of revertants per plate in TA1535 and TA100 strains when tested both in the presence and absence of S9. Positive dose-related increases in the mean number of revertants per plate were observed, in the absence of S9, with TA1535, from 200 to 400 µg/plate, and with TA100, from 100 to 400 µg/plate. The maximum increases obtained were 12.7-fold in TA1535 and 4.2-fold in TA100. Besides, 2.9-fold increases in the mean number of revertants per plate were observed in TA1535, at 300 and 400 µg/plate, with S9 and a 1.6-fold increase in TA100 at 300 µg/plate, with S9. No increases in the mean number of revertants per plate were observed with any of the remaining tester strain/activation condition combinations.

Table 35: Mutagenicity assay Results (Initial test) (Anonymous 35, 1999)

With S9 mix (Rat liver)								
		Revertants/plate				Background Lawn*	Revertants/plate	Background Lawn*
Test article	Dose/ plate (µg)	TA98 Mean (S.D.)	TA100 Mean (S.D.)	TA1535 Mean (S.D.)	TA1537 Mean (S.D.)		WP2uvrA Mean (S.D.)	
Vehicle control		24 (4)	101 (6)	11 (7)	7 (1)	1	12 (8)	1
1.3-D	10	26 (4)	110 (13)	10 (1)	5 (3)	1	13 (2)	1
	33.3	27 (2)	110 (5)	12 (8)	6 (3)	1	16 (5)	1
	100	26 (4)	136 (21)	16 (5)	5 (1)	1	18 (7)	1
	333	20 (1)	160 (8)	33 (4)	4 (2)	1	16 (3)	1
	1000	6 (1)	53 (35)	12 (2)	0 (0)	3	17 (5)	2
	3330	0 (0)	0 (0)	0 (0)	0 (0)	4	0	4
Positive Control**		478 (42)	649 (60)	102 (1)	136 (9)	1	355 (23)	1
No S9 mix								
		Revertants/plate				Background Lawn*	Revertants/plate	Background Lawn*
Test article	Dose/ plate (µg)	TA98 Mean (S.D.)	TA100 Mean (S.D.)	TA1535 Mean (S.D.)	TA1537 Mean (S.D.)		WP2uvrA Mean (S.D.)	
Vehicle control		19 (3)	96 (12)	7 (2)	4 (1)	1	17 (1)	1
1.3-D	3.33	19 (5)	77 (11)	8 (1)	10 (3)	1		
	10	14 (1)	87 (11)	11 (4)	7 (3)	1	14 (2)	1
	33.3	13 (10)	80 (13)	8 (2)	6 (2)	1	14 (3)	1
	100	12 (1)	91 (31)	18 (6)	6 (2)	1	15 (3)	1
	333	17 (3)	208 (13)	46 (8)	3 (1)	1	16 (2)	1
	1000	4 (5)	38 (34)	2 (3)	0 (0)	3	15 (3)	3
	3330						0 (0)	5
Positive Control***		243 (12)	674 (32)	659 (14)	1894 (94)	1	533 (82)	1

Table 36: Mutagenicity assay Results (Re-test) (Anonymous 35, 1999)

With S9 mix (Rat liver)								
		Revertants/plate				Background Lawn*	Revertants/plate	Background Lawn*
Test article	Dose/ plate (µg)	TA98 Mean (S.D.)	TA100 Mean (S.D.)	TA1535 Mean (S.D.)	TA1537 Mean (S.D.)		WP2uvrA Mean (S.D.)	
Vehicle control		24 (3)	99 (7)	13 (5)	7 (1)	1(TA98 & 1537) 1(TA100 & 1535)	14 (3)	1
1.3-D	10	31 (4)	118 (8)	11 (5)	7 (1)	1(TA98 & 1537) 1(TA100 & 1535)	11 (2)	1
	33.3	23 (2)	101 (8)	19 (5)	7 (1)	1(TA98 & 1537) 1(TA100 & 1535)	13 (2)	1
	100	28 (3)	108 (20)	16 (3)	7 (3)	1(TA98 & 1537) 1(TA100 & 1535)	14 (2)	1
	200		111 (19)	30 (6)		1(TA100 & 1535)		
	300		156 (13)	38 (7)		1(TA100 & 1535)		
	333	28 (6)			6 (2)	1(TA98 & 1537)	22 (4)	1
	400		120 (24)	38 (10)		1(TA100 & 1535)		
	500		92 (24)	20 (9)		1(TA100 & 1535)		
	1000	14 (1)	0 (0)	1 (2)	3 (4)	3(TA98 & 1537) 3(TA100 & 1535)	17 (3)	2
	3330	0 (0)	0 (0)	0 (0)	0 (0)	5(TA98 & 1537) 4(TA100 & 1535)	0 (0)	5
Positive Control**		593 (11)	882 (35)	119 (12)	120 (7)	1(TA98 & 1537) 1(TA100 & 1535)	251 (30)	1
No S9 mix								
		Revertants/plate				Background Lawn*	Revertants/plate	Background Lawn*
Test article	Dose/ plate (µg)	TA98 Mean (S.D.)	TA100 Mean (S.D.)	TA1535 Mean (S.D.)	TA1537 Mean (S.D.)		WP2uvrA Mean (S.D.)	
Vehicle control		21 (5)	76 (7)	10 (2)	4 (3)	1(TA98 & 1537) 1(TA100 & 1535)	13 (3)	1
1.3-D	3.33	20 (6)	92 (13)	8 (2)	3 (1)	1(TA98 & 1537) 1(TA100 & 1535)		
	10	19 (6)	97 (9)	11 (2)	5 (1)	1(TA98 & 1537) 1(TA100 & 1535)	11 (2)	1
	33.3	25 (6)	104 (7)	21 (9)	6 (2)	1(TA98 & 1537) 1(TA100 & 1535)	15 (4)	1
	100	21 (3)	162 (45)	28 (3)	4 (3)	1(TA98 & 1537) 1(TA100 & 1535)	14 (5)	1
	200		212 (9)	49 (8)		1(TA100 & 1535)		
	300		298 (11)	83 (28)		1(TA100 & 1535)		
	333	23 (3)			4 (2)	1(TA98 & 1537)	14 (2)	1
	400		318 (60)	127 (27)		1(TA100 & 1535)		
	500		299 (41)	108 (46)		1(TA100 & 1535)		
	1000	25 (5)	82 (9)	4 (7)	1 (2)	3(TA98 & 1537) 3(TA100) 4(1535)	19 (5)	2
	3330						0 (0)	4
Positive Control***		280 (18)	826 (11)	653 (36)	1665 (117)	1(TA98 & 1537) 1(TA100 & 1535)	365 (12)	1

** TA98 benzo(a)pyrene 2.5 µg/p1ate
TA100 eminoenthracene 2.5 µg/p1ate
TAl535 2-aminoenthracene 2.5 µg/plate
TAI537 2-aminoenthracene 2.5 µg/plate
WP2uvrA 2-aminoenthracene 25.0 µg/plate

*** TA98 nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TAU35 sodium azide 2.0 µg/plate
TAI537 ICR-191 2.0 µg/plate
WP2uvrA 4-nitroquinoline-N-oxide 0.4 µg/plate

* Backaround Lawn Code.:
1 – normal, 2 – slightly reduced, 3 – moderately reduced, 4 - extremely reduced, 5 – absent, 6 - obscured by precipitate.
s.p. - .slight precipitate, (requires hand count)
mp - moderate precipitate, (required hand count)
hp - heavy precipitate
NC – no count due to technical error. Am aliquot of tester strain was not added to the plate.

1,3-D caused positive increases in the mean number of reverse mutations with tester strains TA1535 (6.6-fold and 12.7-fold) and TA100 (2.2-fold and 4.2-fold) in the absence of an exogenous metabolic activation system and was weakly mutagenic for TA1535 and equivocal for TA100 when tested in the presence of S9. 1,3-D did

not cause positive increases in the mean number of reverse mutations with any of the remaining tester strain/activation conditions.

In 2009, 1,3-D was again tested in a bacterial mutagenicity study using pre-incubation method in the tester strains *S. typhimurium* strains TA100 and TA1535 (Anonymous 33, 2009). The assay was conducted with eight dose levels of test article in both the presence and absence of purified rat liver microsomes along with concurrent vehicle and positive controls using three plates per dose. The doses tested were 10.0, 33.3, 66.7, 100, 333, 667, 1000, and 2000 µg/plate (in vehicle DMSO) in both the presence and absence of an exogenous metabolic activation system. Under the conditions of this study (Table 37), 1,3-D caused a positive increase (i.e. at least a 2- or 3-fold increase in TA 100 and TA1535, respectively) in the mean number of revertants per plate with both of the tester strains in the presence and absence of an exogenous metabolic activation system prepared from purified rat liver microsomes.

Table 37: Assay Results/Summary data (Anonymous 33, 2009)

	Dose (µg)	Mean revertants/plate with standard deviation				Background Lawn
		TA100		TA1535		
		Mean	S.D.	Mean	S.D.	
Microsomes: Rat liver						
Vehicle control		104	5	16	1	1
1,3-D	10.0	92	4	13	3	1
	33.3	107	8	11	7	1
	66.7	119	13	19	8	1
	100	137	9	21	5	1
	333	172	19	39	5	1
	667	260	24	69	7	1
	1000	248	12	73	9	1/2[a]
	2000	109	7	29	5	1
Positive control **		1172	48	187	16	1
Microsomes: None						
Vehicle control		97	5	14	5	1
1,3-D	10.0	92	8	12	3	1
	33.3	104	14	16	4	1
	66.7	102	10	15	2	1
	100	106	10	13	2	1
	333	160	14	39	8	1
	667	313	28	79	1	1
	1000	341	85	84	17	2
	2000	73	8	13	6	3
Positive control ***		939	21	742	10	1

** TA100 2-aminoanthracene 3.3 µg/plate
TA1535 2-aminoanthracene 3.3 µg/plate
*** TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
* Background Lawn Evaluation Codes:
1 = normal 2 = slightly reduced 3 = moderately reduced 4 = extremely reduced 5 = absent 6 = obscured by precipitate
[a] The first entry is the lawn evaluation for tester strain TA100. The second entry is the lawn evaluation for tester strain TA1535

Anonymous 31 (2004) aimed to evaluated species differences in the i*n vitro* activation of 1,3-D in a bacterial mutagenicity assay on just one strain, *S. typhimurium* (TA1535) following four activation conditions: 1) without exogenous metabolic activation, 2) with purified microsomes, 3) with S100 (cytosol) and glutathione (GSH), and 4) with purified microsomes, S100, and GSH. S100 and microsomes from each of the following species were used: 1) rat, 2) mouse, and 3) human. The study was performed using the preincubation exposure method. The mutagenic activity of 1,3-D was evaluated using eight doses in the vehicle ethanol (2000, 1000, 667, 333, 100, 66.7, 33.3, and10.0 µg per plate) along with concurrent vehicle and positive controls and using each of the four activation conditions described above with either rat, mouse, or human S100 and microsomes. Positive increases in the mean number of revertants per plate were observed in the absence of exogenous metabolic activation (7.3-fold) and in the presence of rat (6.3-fold), mouse (8.3-fold), and human (6.1-fold) microsomes. For all three species, when S100 and GSH were added to the preincubation mix, and when microsomes, S100, and GSH were added to the preincubation mix, no positive increases in the mean number of revertants per plate were observed. The results with 1,3-Dichloropropene are summarized as follows:

Table 38: Assay summary data – single tester strain S. typhimurium (TA1535) (Anonymous 31, 2004)

	Activation conditions			
Species	None	Microsomes	S100 and GSH	Microsomes, S100, and GSH
Without Activation	+ (7.3)			
Rat		+ (6.3)	- (1.2)	- (1.4)
Mouse		+ (8.3)	- (1.2)	- (2.6)
Human		+ (6.1)	- (1.1)	- (1.8)
+ = positive response, - = Negative response, ( ) = Maximum fold induction				

The data indicate that in the absence of exogenous metabolic activation and in the presence of rat, mouse, or human microsomes, mutagenic activity was observed with 1,3-dichloropropene. For all three species, when S100 and GSH were added to the preincubation mix, and when microsomes, S100, and GSH were added to the preincubation mix, no mutagenic activity was observed with 1,3-dichloropropene..

The Anonymous 39 (1989) bacterial mutagenicity study is considered as additional information only, as it describes a modification of the bacterial mutagenicity test, with significant deviations from OECD TG 471. The aim of the study was to determine the mutagenicity of the urine of mice repeatedly administered 1,3-dichloropropene (1,3-D) via gavage, and of several compounds which have been identified as urinary excretion products of 1,3-D, or are theorized to be potential excretion products of 1,3-D, in order to demonstrate that; 1) a mutagenic metabolite of 1,3-D is responsible for hyperplasia observed in the urinary bladder transitional epithelium of mice repeatedly exposed to 1,3-D via gavage or inhalation; 2) to examine the potential of rat and mouse renal enzymes to activate the cysteine conjugate of 1,3-D to a mutagen *in vitro*; and 3) to determine the *in vitro* activity of rat and mouse hepatic and renal beta-lyase using the cysteine conjugate of 1,3-D as a substrate. 1,3-D excretion products and putative metabolites of 1.3-D were added to incubation mixtures as DMSO (cysteine, N-acetylcysteine, and thioglycolic acid conjugates) or distilled water (sulfoxide/sulfone conjugate and disulfide) solutions dependent on their solubilities. The metabolites investigated were:

- N-acetyl-S-(3-chloroprop-2-enyl) cysteine (N-acetylcysteine conjugate) (Mouse & Rat metabolite) (solvent DMSO)
- 3-chloroprop-2-enyl-S-cysteine (cysteine conjugate) (Putative metabolite) (Solvent DMSO)
- N-acetyl-S-(3-chloroprop-2-enyl) cysteine sulfoxide/sulfone (sulfoxide/sulfone conjugate) (Mouse and Rat metabolite) (Solvent distilled water)
- S-(3-chloro-2-propenyl) mercaptoacetic acid (thioglycolic acid conjugate) (Mouse metabolite) (Solvent DMSO)
- bis-(3-chloro-2-propenyl) disulfide (disulfide) (Mouse metabolite) (tested -S9 only) (Solvent distilled water)

Urine, collected from mice repeatedly dosed with 100 mg/kg/day 1,3-D, and the disulfide metabolite of 1,3-D were not mutagenic, under the conditions of this study. However, N-acetylcysteine, sulfoxide/sulfone, thioglycolic acid and cysteine conjugates of 1,3-D were mutagenic for TA100 in the absence of metabolising enzymes, although the maximum increase in the number of revertants induced by N-acetylcysteine conjugate was only 2.9-fold control value. The sulfoxide/sulfone and cysteine conjugates of 1,3-D also caused an increase in the number of TA98 revertants, albeit smaller than that observed with TA100. While these 1,3-D conjugates were found to be mutagenic at relatively high concentrations (5-10 mg/plate), it is estimated that, based upon pharmacokinetic data, their concentration in the urine of either sex of mice dosed with 1.3-D in this study would not have been high enough to expect a positive urine assay. Further, the cysteine conjugate of 1,3-D was found to be non-mutagenic in the presence of a kidney homogenate and to be a relatively poor substrate of hepatic and renal beta-lyase *in vitro*, suggesting a lack of metabolism to a more reactive thiol intermediate theorized to occur in mice. Results of this study do not rule out the possible metabolic activation of the cysteine conjugate of 1,3-D to a toxic compound *in vivo,* possibly within the bladder epithelium itself. However, these data do not support the theory that a highly mutagenic urinary excretion product of 1,3-D is directly responsible for the observed toxicity of 1,3-D in the urinary bladder of mice. This, however, does not rule out the possibility that certain other characteristics of these compounds (e.g. chemical irritancy) and species-specific tissue sensitivities are involved.

In the ninth mutagenicity study (Anonymous 42, 1978), the addition of cis-1,3-dichloropropene to cultures of Salmonella TA100 and TA1535 resulted in dose-dependent increases in the frequencies of reversion was investigated. Both of these strains are sensitive to the induction of base-pair substitutions and the results obtained in the most sensitive strain TA100 are shown in Table below.

Table 39: Reversion rate in Salmonella typhimurium TA100 after treatment with cis-1,3-dichloropropene in the plate incorporated assay

Concentration (µg/plate)	- S9	+ S9
0	11*	20*
20	24	31
100	101	113
500	217	242
2000	411	707

* results are expressed as revertants/plate after subtraction of the spontaneous revertants

There was some evidence for an additional increase in mutation frequency when the 9000 g supernatant fraction from rat liver was incorporated in the test system.

In a series of experiments designed to study the kinetics of the initial conjugation reaction, cis-1,3-dichloropropene was incubated with rat liver 10000 g supernatant in the presence and absence of added glutathione.

Table 40: Percentage substrate recovery

Substrate incubated with	Percentage substrate recovered after 10 min
Buffer	Ca. 90
Buffer + GSH	Ca. 90
Buffer + 10000 g supernatant	72
Buffer + 10000 g supernatant + GSH	0

These results show that the spontaneous reaction between glutathione and cis-1,3-dichloropropene proceeded at an immeasurably slow rate. However, the 10000g supernatant fraction from rat liver was extremely efficient in catalysing the biotransformation of cis-1,3-dichloropropene. This efficient catalysis was however only observed when the concentration of glutathione was adjusted to a normal physiological value. The normal physiological concentration of glutathione in rat liver ranges from 4.4 to 7.64 mmol/g and is greatly reduced as a consequence of dilution and oxidation during the preparation of 10000 g supernatants. The current results demonstrate that the low concentration of a endogenous glutathione present in the 10000 g supernatant from rat liver was severely rate-limiting.

The results of the effect of glutathione on the bacterial mutagenicity of cis-1,3-dichloropropene are as below.

Table 41: Effect of glutathione on the bacterial mutagenicity of cis-1,3-dichloropropene

Concentration (µg/plate)	S9	- 5 mM glutathione	+ 5mM glutathione
0	Absent	52*	37*
10		0	0
25		0	0
50		0	0
100		33	0
250		71	0
500		132	4
0	Present	38*	35*
10		7	6
25		17	0
50		24	1
100		62	11
250		118	33
500		185	81

* results are expressed as revertants/plate after subtraction of spontaneous revertants

Cis-1,3-Dichloropropene induced a dose-related increase in the reverse mutation rate of S. typhimurium TA100. The inclusion of the 9000 g supernatant from the livers of Arachlor-treated rats resulted in small but consistent increases in mutation frequency. The addition of a normal physiological concentration (5 mM) of glutathione to the soft agar overlay completely eliminated the supposedly direct mutagenic activity of cis-1,3-dichloropropene in the absence of the 9000g supernatant from rat liver and markedly reduced the activity in the presence of this fraction.

In the tenth study (Anonymous 43, 1978), cis-1,3-Dichloropropene was tested for mutagenic potential in strains of Salmonella typhimurium TA98, TA 100, TA 1535 and TA 1538. As part of a study, the influence of a physiological concentration of glutathione on the mutagenicity of cis-1,3-dichloropropene was investigated in the most sensitive bacterial strain (TA100).

The addition of cis-1,3-dichloropropene to cultures of TA100 and TA1535 resulted in an increase in the reversion frequency. Mutation frequencies were marginally higher in the presence of S-9 than in its absence. Cis-l.3-dichloropropene also induced a marginal increase in mutation-in strain TA98, suggesting the induction of frameshift mutations by this isomer. In the S. typhimurium TA1538 did not show an increase in mutation, but there was a significant reduction in the growth of the background lawn and S typhimurium TA100 was selected as the most sensitive bacterial strain for use in the further experiments.

Cis-1.3-Dichloropropene induced a dose-related increase in the reverse mutation rate of S. typhimurium TA100. The inclusion of S-9 resulted in small but consistent increases in mutation frequencies. The addition of 5 mM glutathione to the soft agar overlay eliminated the mutagenic activity completely in the absence of the rat liver microsomal fraction and markedly reduced the activity in the presence of this fraction.

Cis-1,3-Dichloroprene induced a dose-related increase in the reverse-mutation rate of Salmonella typhimurium TA1535, TA98 and TA100 both in the presence and absence of a rat liver microsomal activation system. The incorporation of 5 mM glutathione afforded protection against the mutagenicity of cis-1,3-dichloropropene up to a concentration of 250 µg per plate both in the presence and absence of a rat liver microsomal activation system.

The eleventh study (Anonymous 44, 1990) was GLP -compliant and conducted to a test method similar to OECD Guideline 471.The mutagenic activity of cis-1,3 -dichloropropene was investigated in agar layer cultures of selected bacterial tester strains of Salmonella typhimurium and Escherichia coli. Assays were performed in both the presence and absence of S9 microsomal fraction obtained from a liver homogenate of rat pre-treated with Aroclor 1254. It was concluded that cis-1,3 -dichloropropene was direct-acting bacterial mutagen under the experimental conditions employed. A mutagenic dose of the test material was tested in the presence of a range of concentrations of glutathione. Glutathione reduced the mutagenicity of the test material in the presence and absence of S9 fraction.

Mammalian gene mutation

1,3-D has been tested in an *in vitro* mammalian cell gene mutation test using the *Hprt* gene in Chinese hamster ovary cells (HPRT test) (Anonymous 36, 1986). The test is generally in line with the current OECD TG 476 (2016). The study was conducted using a Chinese hamster ovary cell line designated CHO-K_1-BH_4 with or without the metabolic activation system S9 derived from livers of male Sprague-Dawley rats induced with Arochlor 1254. Appropriate positive controls were included. In the first non-activated experiment, 1,3-D resulted in relative survival values of approximately 105%, 55%, 18%, 3% and <1% of that of the control cultures at 50, 100, 150, 200 and 250 µM, respectively. An apparent increase in mutation frequency was observed at 200 and 250 µM. However, the biological significance of this observation is doubtful due to the extreme toxicity observed at these concentrations. Positive control gave a satisfactory response. In the second non-activated experiment, 1,3-D resulted in relative survival values of approximately 122%, 37%, 12%, 11% and 9% of that of the control cultures at 50, 100, 150, 200 and 250 µM, respectively. No increase in mutation frequency was observed at any concentration. Positive controls gave a satisfactory response. In the third non-activated experiment, the maximum decrease in relative survival, observed at 200µM, was approximately 70%. The reason for the lesser degree of cell killing observed is not clear. 1,3-D induced no significant increased mutation frequencies. Positive controls gave a satisfactory response. In the activation assay, 1,3-D resulted in

relative survival values of approximately 98%, 69%, 68%, 48% and 14% of that of the control cultures at 50, 100, 125, 150 and 200 µM, respectively. 1,3-D induced no significant increased mutation frequencies. Positive controls gave a satisfactory response. In conclusion, 1,3-D was not mutagenic in the CHO/HGPRT assay.

Mammalian Chromosome Aberration

Anonymous 38 (1988) evaluated 1,3-D for Chromosome aberration in cultured mammalian cells, using Chinese hamster lung (CHL) cells ±S9 derived from livers of male Sprague-Dawley rats induced with Aroclor 1254. Appropriate positive controls were included. Dose levels for the chromosome aberration assay were selected following a preliminary toxicity test based upon growth after treatment relative to solvent control. 1,3-D was tested at concentrations of 34.68, 69.36, 138.7, 194.2 and 277.5 µg/ml (without S9) and 17.34, 34.68, 69.36, 138.7 and 277.5 µg/ml (with S9) in solvent DMSO, in two independent mutagenicity assays carried out with concurrent negative (untreated and solvent treated cells) and positive controls. In the first assay without S9, the frequencies of aberrant structural metaphases (excluding gaps), induced by 1,3-D at 34.68, 69.36, 138.7, 194.2 and 277.5 µg/ml, were 1, 0.5, 6, 16 and 29% (after 24 h treatment) and 2.5, 5.5, 8.5, 14 and 14% (after 48 h treatment); and the frequencies of polyploid metaphases were 0.5, 0.5, 1.5, 1.5 and 1.5% (after 24 h treatment) and 0, 3, 8.5, 12 and 12.5% (after 48 h treatment). In the confirmatory assay, conducted at the same concentrations, the frequencies of aberrant structural metaphases (excluding gaps) were 0.5, 0, 1, 3.5 and 21.5% (after 24 h treatment) and 0.5, 0.5, 0,5, 1.5 and 10.5% (after 48 h treatment); and the frequencies of polyploid metaphases were 0, 0.5, 0.5, 0.5 and 1% (after 24 h treatment) and 0.5, 0, 1.5, 3.5 and 12% (after 48 h treatment). In both assays, the increases in the frequency of aberrant structural metaphases, after 24 and 48 h treatment, as well as the increases in the frequency of polyploid metaphases, after 48 h treatment, were concentration dependent with values >10% at the one or two highest concentrations tested. Positive controls gave a satisfactory response in both assays. Toxicity, as a reduction of ≥50% in mitotic index, was observed in the first assay at 277.5 µg/ml, after the treatment of 24 h, and at 138.7 µg/ml, after the treatment of 48 h; and in the confirmatory assay, at 138.7 and 277.5 µg/ml, after the treatment of 48 h.

In the first assay with S9, the frequencies of aberrant structural metaphases (excluding gaps), induced by 1,3-D at 17.34, 34.68, 69.36, 138.7 and 277.5 µg/ml, were 0, 0, 1.5, 8.5 and 13.1% (after 6 h treatment with 12 h recovery) and 1, 0, 0.5, 12 and 33% (after 6 h treatment with 18 h recovery); and the frequencies of polyploid metaphases were 1, 1, 1, 0 and 0% (after 6 h treatment with 12 h recovery) and 0, 0.5, 1.5, 5 and 1.5% (after 6 h treatment with 18 h recovery. In the confirmatory assay, conducted at the same concentrations, the frequencies of aberrant structural metaphases (excluding gaps) were 0.5, 1, 1.5, 8.5 and 10% (after 6 h treatment with 12 h recovery) and 0, 0, 6.5, 17 and 40.4% (after 6 h treatment with 18 h recovery); and the frequencies of polyploid metaphases, 2, 2, 2, 3, 1.5% (after 6 h treatment with 12 h recovery) and 0, 1, 9.5, 9 and 1.7% (after 6 h treatment with 18 h recovery). In both assays, the increases in the frequency of aberrant structural metaphases after 6 h treatment, with 12- or 18-hours recovery, were concentration dependent with values >10% at the one or two highest concentrations tested; however, the frequencies of polyploid metaphases were <10%. Positive controls gave a satisfactory response in both assays. Cytotoxicity was observed in both assays at 277.5 µg/ml, after 6 h treatment with 12 h recovery, and from 138.7 µg/ml, after 6 h treatment with 18 h recovery. The analysis of 100 metaphases after 6 h treatment with 277.5 µg/ml was not possible, in the first assay (with 12 h recovery) and in the confirmatory assay (with 18 h recovery) because remarkable suppression of cell growth was observed. This study, under the conditions employed, reported an ability of 1,3-D to induce structural chromosome aberrations (with or without S9) and numerical chromosome aberrations (without S9). However, it is not possible to ascertain if adverse changes in pH and/or osmolarity may have contributed to the findings. Furthermore, the highest concentrations analysed for aberrations frequently exceeded the maximum limit of cytotoxicity as recommended by OECD 473 (2016) and therefore, the findings may be due to adverse toxicity rather than direct DNA damage by 1,3-D. Consequently, these results are considered to be of limited reliability.

Kevekordes *et al.* 1996, evaluated 1,3-D in a sister chromatid exchange (SCE) assay. *In vitro* 1,3-D induced increases in SCE-frequencies in human lymphocytes with and without addition of an exogenous metabolizing system. As shown in table 42, the assay with 24 h incubation time showed a mean induction of 11.2 SCE/metaphase up to 16.0 SCE/metaphase for the highest 1,3-dichloropropene concentration (100 µM in DMSO). After 48-h incubation time, the mean of 9.4 SCE/metaphases increased up to 23.0 SCE/metaphase in the highest concentration tested (100 µM) in the presence of a metabolic activation system (rat liver S9),

however, the mean basal SCE rate of 10.5 was elevated up to 15.4 after application of 100 µM 1,3-dichloropropene.

Table 42: Induction of sister chromatid exchanges (SCEs) in cultured human lymphocytes by 1,3-D (Kevekordes *et al.* 1996)

S9	Incubation Time (h)		Concentration of 1.3-D (µmol/L)			
			0	1	10	100
None	24	SCE/metaphase (mean ± S.D)	11.2 ±4.3	8.7 ±3.7	10.1 ±4.0	16.0 ±5.3**
		Proliferation Index	1.8	2.2	2.1	1.6
None	48	SCE/metaphase (mean ± S.D)	9.4 ±3.7	9.1±4.3	9.4 ±3.5	23.0 ±7.0**
		Proliferation Index	1.8	2.1	2.0	2.1
1mg protein /ml culture	2	SCE/metaphase (mean ± S.D)	10.5 ±4.3	10.0 ±4.1	9.1 ±3.6	15.4 ±4.3 **
		Proliferation Index	2.0	1.7	1.8	1.5

It should be noted that OECD TG 479 (in vitro sister chromatid exchange test in mammalian cells) was deleted in 2014 because of a lack of understanding of the mechanism(s) of action of the effect detected by the test, and thus its relevance to human health assessment.

A GLP-compliant study was been conducted in accordance with EPA Guidelines 84-2. It was concluded that cis-1,3-dichloropropene induced chromosome damage in the presence, but not in the absence, of S9 mix in cultured CHO cells. When physiological strength (5 mM) glutathione was added to cis-1,3-dichloropropene-treated cultures in the presence of S9 mix, the extent of chromosome damage was reduced to levels observed in the negative control cultures, under the standard test experimental conditions as described in the guidelines.

The clastogenic potential of trans-1,3-dichloropropene was assessed from assays designed to monitor chromosome damage in Chinese Hamster Ovary (CHO) cells. Cultures were grown in glass bottles and incubated in medium containing the test compound for either 3 hours in the presence of S9 mix or for 24 hours in the absence of S9 mix. Metaphase cells were prepared on glass microscope slides for the analysis of chromosome aberrations 8-, 12- and 24-hours following initiation of compound exposure for the cultures with S9 mix and at 24 hours following exposure for the cultures without S9 mix. From the data generated at the 24 h sample time it was concluded that trans-1,3-dichloropropene induced chromosome damage in the presence of S9 mix; no clastogenic activity was observed in the absence of S9 mix, under the experimental conditions as described in the test guidelines.

DNA Damage and repair

Anonymous 37 (1985) tested 1,3-D in an *in vitro* unscheduled DNA synthesis assay in primary cultures of male CDF Fisher 344 rat hepatocytes. Based on the results of the preliminary cytotoxicity test, eight concentrations of 1,3-D ($1x10^{-6}$, $3x10^{-6}$, $1x10^{-5}$, $3x10^{-5}$, $1x10^{-4}$, $3x10^{-4}$, $1x10^{-3}$ and $3x10^{-3}$ M) in 0.1% DMSO as solvent, were selected for the UDS assay. Negative (0.1% DMSO) and positive (2-AAF at concentrations of 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) controls were included concurrently. At the highest concentration tested ($3x10^{-3}$ M) a precipitate formed upon addition of the test material to the culture medium. Two independent assays were performed where triplicate cultures were treated with each test article and controls in the presence of ^{3}H-thymidine, for 18 hours. After chemical treatment, the cultures were scored for toxicity and then processed for microautoradiography. Fifteen cells on each of two slides were evaluated per dose level. The net number of nuclear grains in treated cells was compared to the negative control. Net nuclear labelling is often observed in control cells but rarely exceeds a mean of 5 grains per nucleus. Therefore, the criteria for a clear positive response was a mean of 6 or more net grains per nucleus, with statistical significance from control at $p< 0.05$ and the presence of a dose response relationship.

As shown in table 43, in the first assay, toxicity was apparent at $1x10^{-5}$ M and greater concentrations. The severity of toxicity decreased in a dose related manner and ranged from complete detachment of the cells from the coverslip at $3x10^{-3}$ M (no cells remained for UDS evaluation) to a granular appearance at $1x10^{-5}$ M. Although data indicated that 1,3-D failed to elicit UDS at any of the concentrations tested, high cytoplasmic grain counts caused an unusually low value for net nuclear grains at several dose levels. In the repeat assay,

toxicity was apparent at $1x10^{-4}$ M and greater concentrations and ranged from a majority of cells detached at $3x10^{-3}$ M to a granular appearance at $1x10^{-4}$ M. Data indicated that 1,3-D failed to elicit UDS at any of the concentration tested and the unusually high cytoplasmic grain counts were not observed. Positive control elicited a significant increase in UDS at concentrations of 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M in the initial assay, and at 10^{-6}, 10^{-5} and 10^{-4} M in the repeat assay, as compared to the negative control.

Table 43: Summary of 1,3-D UDS data (Anonymous 37, 1985)

Treatment	Concentration Moles/litre	Net Nuclear Grains Mean (standard deviation)	
		1st Assay	2nd Assay
Negative Control 0.1% DMSO	0	-3.8 (3.6)	1.4 (3.2)
1,3-D	3 $x10^{-3}$ (precipitated)	Complete toxicity	-0.7 (0.9)*
	1 $x10^{-3}$	-1.0 (1.0)*	0.5 (3.8)*
	3 $x10^{-4}$	-3.5 (3.7)*	-1.2 (2.2)*
	1 $x10^{-4}$	-7.4 (7.3)*	-2.9 (3.5)*
	3 $x10^{-5}$	-12.1 (8.1)*	-2.8 (2.3)
	1 $x10^{-5}$	-5.4 (4.3)	-3.0 (3.0)
	3 $x10^{-6}$	-3.5 (2.8)	-4.1 (3.6)
	1 $x10^{-6}$	-3.1 (3.0)	-2.4 (2.7)
2-AAF^	1 $x10^{-4}$	78.1 (27.1)*^	80.1 (17.2)*^
	1 $x10^{-5}$	56.6 (13.3)*^	86.5 (22.1)*^
	1 $x10^{-6}$	56.3 (20.8)*^	54.6 (12.8)^
	1 $x10^{-7}$	6.4 (5.1)^	4.8 (3.7)

* toxicity

^ Positive response; 6 or more net nuclear grains and statistical significance ($p<0.05$)

1,3-D did not induce UDS in treated rat hepatocytes.

Anonymous 40 (1997) examined the potential of 1,3-D to bind DNA *in vitro* in the presence or absence of potentially activating enzymes and physiological levels of glutathione (GSH). The test substance was ^{14}C-1,3-D at approximately 4.8 µCi and tested at a final concentration of approximately 11 mM. ^{14}C-methyl iodide and ^{14}C-1,2-dichloroethane served as positive controls for directly reactive and metabolically activated DNA-binding chemicals, respectively. S9 was prepared from Arochlor 1254-induced rats. Binding was measured following a prolonged incubation in: a) the absence of enzymes, b) the presence of potentially activating enzymes (S9), and c) the presence of both activating enzymes and added glutathione.
^{14}C-1,3-D was incubated with calf thymus DNA, with or without rat liver S9, for 4 hours at 37°C. Following incubation, the reaction was terminated, and DNA was re-extracted and purified. The purity and concentration of recovered DNA were determined spectrophotometrically. The total radioactivity associated with the recovered DNA was determined using liquid scintillation counting (30-minute counting time) and the numbers of adducts per nucleotide were calculated using the following equation:

$$\text{Adducts}/10^6\ \text{dN} = \frac{(\text{Sample dpm}) \times (333) \times 106}{(\text{Specific Activity of 14C-1,3-dichloropropene}) \times (\mu\text{g DNA in sample counted})}$$

^{14}C-1,3-D was not observed to bind calf thymus DNA in any of the incubation mixtures at a sensitivity of approximately 2-4 adducts/10^6 nucleotides. In contrast, ^{14}C-methyl iodide and ^{14}C-1,2-dichloroethane were observed to bind DNA at levels of approximately 54 and 147 adducts/10^6 nucleotides.

1,3-D did not bind calf thymus DNA *in vitro*, either in the absence or presence of potential activating enzymes, under the conditions of this study.

Summary of *in vitro* data
A total of 11 bacterial mutation assays are available for 1,3-D. The weight of evidence indicates that 1,3-D is capable of inducing gene mutations in bacteria with and without S9. However, 1,3-D did not induce mutation

in CHO cells in an OECD 476 compliant HPRT gene mutation assay, nor did it induce DNA repair in rat hepatocytes treated *in vitro*. 1,3-D was reported to cause chromosome aberrations in CHO cells at concentrations that approached or exceeded acceptable levels of cytotoxicity as well as sister chromatid exchanges. However, 1,3-D did not bind to calf thymus DNA *in vitro*. Therefore, whilst there is evidence that 1,3-D may cause chromosomal damage, the data suggest the mechanism may relate to secondary effects, e.g. cytotoxicity, rather than via direct DNA damage. Observations that the mutagenic effects of 1,3-D in bacteria can be eliminated by co-incubation with glutathione, provides useful evidence regarding the role of glutathione in protecting cells from 1,3-D genotoxicity.

In vivo data

Genotoxicity in somatic tissues

The gene mutation potential of 1,3-D has been investigated in two transgenic rodent assays. One was conducted by oral administration in Big Blue Male rats (Anonymous 24, 2018) and investigated gene mutations in liver and kidney, whilst the other was an inhalation exposure study in Big Blue B6C3F1 male mice (Anonymous 25, 1997) and investigated effects in liver and lung.

In the recently conducted OECD TG 488 transgenic rodent gene mutation assay at the *cII* Locus in male Big Blue® Transgenic F344 Rats (Anonymous 24, 2018), the effect of 1,3-D on mutant frequency at the *cII* gene in liver and kidneys from male transgenic Fischer 344 Big Blue® rats was investigated. Twenty-four F344 homozygous Big Blue® male rats (six/group) were exposed daily to 1,3-D mixed in diet, for 28 consecutive days, at target dose levels of 0 (Control), 12.5, 25 or 50 mg/kg bw/day. The control animals received the basal diet only for the same period. For the positive control, a group of six Big Blue® male rats were administered 20 mg/kg/day of ENU (N-ethyl-N-nitrosourea) formulated in phosphate buffer solution, pH 6.0; at a dose volume of 10 mL/kg. 1,3-D mixed in diet was shown to be stable for at least 7 days when held at room temperature.

All animals survived to their scheduled termination (Day 31), with no toxicologically relevant differences noted in the clinical observations between the control and test substance-treated animals. Total body weight gain (Days 1-31) for the high dose group was 25% lower as compared to the control group, which correlated with lower food consumption values during the last two weeks of exposure.

No statistically significant differences were noted in the mutant frequency (MF) data when test substance-treated groups were compared to the control group, in either tissue. The positive control treatment with ENU produced a greater than 3-fold increase (statistically significant) in MF for both tissues tested, demonstrating the utility of the test system to detect induced mutants following exposure to a known direct-acting mutagen. Available rat ADME data confirm extensive tissue distribution occurs following oral exposure to 1,3-D, thus it is considered that the target tissue have been adequately exposed to 1,3-D and/or its metabolites. It is concluded that 1,3-D does not induce gene mutations in the liver or kidney of exposed rats.

Table 44: Summary of mutant frequency data (Anonymous 24, 2018)

Dose Level (mg/kg/day)	Liver Mean ± SD (x 10^{-6})	Kidneys Mean [Median] ± SD (x 10^{-6})
0 (placebo control)	32.2 ± 4.2	19.1 [20.6] ± 6.5
12.5	23.4 ± 6.7	22.4 ± 5.9
25	21.5 ± 5.5	25.4 ± 12.8
50	33.4 ± 13.7	19.8 ± 6.4
20 mg/kg ENU[a]	117.0** ± 41.1	104 [108.7*] ± 15.0

[a] = Days 1, 2, 3, 12, 19 and 26 only; SD = Standard Deviation

* = Statistically significant (Kruskal-Wallis test, p = 0.009) compared to Group 1

** = Statistically significant (One-way ANOVA, p < 0.001) compared to Group 1

In the transgenic rodent gene mutation assay conducted by Anonymous 25 (1997), male B6C3F1 hybrid mice (also known as Big Blue mice), 5 per group, were exposed to 1,3-D vapours by inhalation at targeted concentrations of 0 (negative control), 10, 60 and 150 ppm for 2 weeks (6 hours/day, 5 days/week). Following exposure, the mice were maintained under standard laboratory conditions for 17 days. This period, referred to

as the expression period, was intended to allow the fixation of any induced DNA-lesions into mutations. Only liver and lung tissues from the negative control and the highest exposure group (150 ppm) were used to screen for *lacI* mutations. Tissues from other animals were stored at -80°C for possible future analysis. Tissue samples (lung and liver), stored at approximately –80°C and obtained from Big Blue B6C3F1 male mice treated by gavage with five daily doses of diethylnitrosamine were used as positive controls.

All animals survived treatment until their scheduled necropsies. At 150 ppm, decreased activity was observed on test days 3-5 and 8-15. All animals appeared normal at all other times. There were no remarkable in-life cage-side observations in mice in the lower exposure groups. There were no remarkable differences in body weights observed throughout the 2 weeks of exposure or the 2 weeks of expression time. The frequencies of *lacI* mutations in the lung and liver tissues of the 150 ppm 1,3-dichloropropene-exposed group were found to be comparable to the negative control frequencies, while, marked increases in the mutant frequency were observed in the positive control tissue samples, demonstrating the sensitivity of the test system. Analysis of the low and intermediate exposure groups was not conducted as the high exposure group was concluded to be clearly negative for mutant induction in both tissues.

Table 45: Summary of mutant frequency data (Anonymous 25, 1997)

Exposure (ppm)	Liver Mean ± SD (x 10^{-5})	Lung Mean ± SD (x 10^{-5})
0 (Air control)	10.3 ± 4.2	13.3 ± 3.5
150	9.9 ± 3.9	11.2 ± 1.9
Positive control	134.7	59.8

Although the duration of exposure in this study is shorter than that suggested in the current version of OECD 488 (2013) it is consistent with the recommendations available at the time the study was conducted (Heddle *et al.* 2000 and Thybaud *et al.* 2003). Furthermore, the design used is adequately sensitive for the tissues examined as described by Lambert *et al*., 2005[2] and provides further supporting evidence that 1,3-D does not cause gene mutations in somatic tissues *in vivo*.

The clastogenic / aneugenic activity of 1,3-D was investigated in four *in vivo* micronucleus tests; three in mice (Anonymous 26, 1985; Shelby *et al.*, 1993; Kevekordes *et al.*, 1996) and one in rats (Ghia *et al.*, 1993).

In Shelby *et al.* (1993) micronucleus tests with 1,3-D were conducted in two independent laboratories using the same study design and dose levels. The maximum dose selected for micronucleus test was 125 mg/kg based on mortality observed in the dose determination study (100% at 250 mg/kg and 20% at 125 mg/kg). For the initial micronucleus test, groups of 5 mice (7 at the highest dose group) were injected i. p. on three consecutive days with either 1,3-D (31, 62.5 and 125 mg/kg), a weakly active dose of the positive control, or the vehicle. Mice were sacrificed 24-h after the third treatment. Bone marrow smears (two slides/mouse) were prepared. For each animal, slides were evaluated for the number of MNPCE among 2000 PCE and for the % PCE among 200 erythrocytes. To determine whether a specific treatment resulted in a significant increase in MNPCE, the number of MNPCE were pooled within each dose group and analysed by a one-tailed trend test. The %PCE data were analysed by an analysis of variance (ANOVA) test based on pooled data. Pairwise comparisons between each group and the concurrent solvent control group was by an unadjusted one-tailed Pearson chi-squared test. This initial test was negative (p-values for all pairwise comparison in both laboratories were ≥0.1723 and all mean MNPCE were within the historical range reported by the respective laboratory), although there was a difference in linear trend between the laboratories (0.068 vs 0.910). The positive control gave a satisfactory response, and the % PCE observed in the groups treated with 1,3-dichloropropene did not suggest depressed erythropoietic activity.

Table 46: Summary Micronucleus Data (Bone Marrow--Three Exposure) (Shelby *et al.*, 1993)

Lab	Trend P value	Dose (mg/kg)	MN-PCE/1000 (# of animals)	Pair-wise	Survival	%PCE

[2](Mutation Research 590 (2005) 1–280)

Lab	Trend P value	Dose (mg/kg)	MN-PCE/1000 (# of animals)	Pair-wise	Survival	%PCE
1	**0.068**	0	2.40 ± 0.49 (5)		5/5	62.9
		31	1.50 ± 0.27 (5)	0.9254	5/5	60.0
		62.5	1.50 ± 0.16 (5)	0.9254	5/5	64.0
		125	3.10 ± 0.58 (5)	0.1723	7/7	55.8
2	**0.910**	0	3.60 ± 0.91(5)		5/5	55.7
		31	3.90 ± 0.53 (5)	0.3643	5/5	62.3
		62.5	2.70 ± 0.46 (5)	0.8720	5/5	62.3
		125	2.80 ± 0.12 (5)	0.8417	6/6	57.5

To investigate the results further, two single-exposure micronucleus tests were conducted with harvest times of 24- and 48-h post-exposure – the number of animals per group were not reported. The first 24-h harvest test, using doses of 50, 100 and 200 mg/kg, was positive by trend analysis (P=0.032), but no dose groups were significantly elevated and all mean MNPCE frequencies were within the historical control range and below the reported mean historical control value. A second test, with 24 h harvest and doses of 100, 150 and 250 mg/kg, showed no evidence of any increase in MNPCE. Two tests, using a 48-h harvest time, with doses of either 100 or 200 mg/kg (the first test), and 150 or 250 mg/kg (the second test), were positive by trend analysis, with P = 0.006 and 0.001, respectively (although the relevance of a trend analysis on only two dose groups is questionable). In the first test, 200 mg/kg, and in the second test, both 150 and 250 mg/kg induced significant increases in MNPCE, although these MNPCE were highly comparable with the mean historical control value and the historical control range. Furthermore, the MNPCE frequencies observed at 48 h in all treated animals were lower than or similar to control values observed in the repeat dose treatments.

Table 47: Summary Repeat Tests Using Higher Doses & Single Exposure (Shelby *et al.*, 1993)

Sample time (h)	Trend P	Dose (mg/kg)	MN/PCE ±SE	Pairwise P
24	0.032	0	1.00 ± 0.16	
		50	1.10 ± 0.19	0.4136
		100	1.00 ± 0.35	0.5000
		200	1.79 ± 0.26	0.0579
24	0.104	0	0.90 ± 0.40	
		100	1.70 ± 0.34	0.0582
		150	1.00 ± 0.27	0.4092
		250	1.70 ± 0.72	0.0582
48	0.006	0	0.90 ± 0.29	
		100	1.00 ± 0.22	0.4092
		200	2.07 ± 0.40	0.0122
48	0.001	0	0.70 ± 0.20	
		150	2.00 ± 0.22	0.0061
		250	2.50 ± 0.69	0.0007

1,3-D was reported by the study authors as positive but only when tested at doses of 150 mg/kg or greater, after a single exposure and 48-h harvest time. Evaluation of the data using criteria defined in OECD 474 (2016) would suggest an equivocal conclusion is more appropriate for the single exposure, 48 h sample time data, but a clear negative result for all other conditions.

The second MN study showing a positive response, is that of Kevekordes *et al.* (1996). 1,-3-D is reported to significantly increase the frequencies of MNPCE in bone marrow cells of female mice from 3.3 MN/1000 PCE to 15.3 MN/1000 PCE at a dose of 187 mg/kg bw. Cyclophosphomide was used as the positive control and produced the expected increase in MN frequency (75.8 MN/1000 PCE). This study deviates from the current OECD test guideline for the mammalian erythrocyte micronucleus test (OECD TG 474, 2016) in a number of ways. Samples of bone marrow were only taken once at 48 hours, when they should be taken at least twice (from independent groups of animals), starting not earlier than 24 hours after treatment, but not extending beyond 48 hours after treatment with appropriate interval(s) between samples. Only 1000 polychromatic erythrocytes per animal were scored for the presence of micronuclei, instead of the recommended 4000, and importantly, only 1 animal of each sex was used in the solvent control groups. In this study, the female mouse data suggest a positive response, whereas the males showed a dose-related decrease in MN with increasing

doses up to 280 mg/kg. The high doses were described as the 'highest tolerated dose' and the top dose was lethal to 1/4 females.

Table 48: Induction of micronucleated polychromatic erythrocytes in bone-marrow cells in NMRI mice after treatment with 1,3-dichloropropene (Kevekordes *et al.*, 1996)

Dose (mg/kg)	Number of Animals	MN/10³ PCE ± SD	PCE/NCE ± SD
Male mice			
0	1	2.0	0.39
600 CP (Positive control)	1	85.0	0.10
140	4	1.60 ± 0.14[a]	0.42 ± 0.10
280	4	0.40 ± 0.42	0.64 ± 0.18
Female mice			
0	1	3.3	1.35
450 CP (positive control)	1	75.8	0.82
187	4	15.3 ± 1.88[c]	1.56 ± 0.19
234	4/3[b]	14.9 ± 1.45[c]	1.85 ± 0.18

[a] Mean and standard deviation
[b] Number of animals that survived
[c] Mann-Whitney-Wilcoxon test (P<0.01)
CP – cyclophosphamide; MN – micronuclei; PCE – polychromatic erythrocytes; NCE – normochromatic erythrocytes.

However, the use of only one animal of each sex in the solvent control groups is not accepted practice, nor is it supported by OECD or other test guidelines. This deficiency in the protocol renders the entire test unacceptable because variability among replicate animals in the control could be sufficient to change the evaluation of the test chemical results. Furthermore, there was no explanation provided regarding the inconsistency in the observed MNPCE response between male and female mice nor the very unusual PCE:NCE ratios for both sexes. In healthy mice of the age used (7-12 week) erythropoiesis should be in steady state and consequently a PCE:NCE ratio approximating to 1 would be expected. The ratio seen in the control male was markedly below 1 whilst that in the female was greater than 1. Furthermore, the treated females showed a dose related increase in PCE:NCE ratio suggesting significant erythropoiesis, which could explain the apparent increase in MNPCE. These discrepancies add to the weight of evidence that these data are unreliable and should not be used for 1,3-D evaluation.

In the eighth study (Anonymous 47, 1999), the test substance trans-1,3-Dichloropropene (Trans-D) was examined in a bone marrow micronucleus test in mice. Test animals were exposed to the test substance through inhalation during 4 hours at three exposure levels: 150, 300 and 600 ppm (135, 268 and 535 ppm, corrected for the presence of the stabiliser), with 5, 5 and 10 animals per level, respectively. Mice of the negative control group (10 males) were treated in a similar way at 0 ppm exposure level. A positive control group (5 males) was concurrently given a single intraperitoneal dose of the mutagen mitomycin C (0.75 mg/kg-bw). At 24 hours after start of the exposure, 5 negative controls, 5 test animals per exposure group and the 5 positive controls were sacrificed. At 48h after start of the exposure, 5 negative controls and the 5 test animals of the highest exposure group were sacrificed. Bone marrow cells were collected and pooled from both femurs of all animals and processed into smears for microscopic examination. At both sacrifice times the incidences of micronucleated polychromatic erythrocytes (MPE) per 2000 polychromatic erythrocytes (PE) in mice treated with the test substance Trans-D were not statistically significantly higher than those found in the negative controls. Therefore, the study indicates that treatment with the test substance Trans-D did not result in genotoxicity to bone marrow cells. The incidence of micronuclei in the positive control group was significantly increased compared to the negative control. Adequate exposure of the target cells was demonstrated by a statistically significant negative trend in the number of polychromatic erythrocytes per number of erythrocytes in relation to the exposure level in mice treated with the test substance Trans-D. It was concluded that the test substance Trans-D did not produce micronuclei in polychromatic erythrocytes in mice under the conditions used in this study.

In contrast to the Kevekordes *et al.* (1996) study, the study of Anonymous 26 (1985) was reported to be negative for micronucleated polychromatic erythrocytes in both male and female CD®-1 (ICR) BR mice treated with 1,3-D, compared to negative controls. In this micronucleus test, 1,3-D (38, 115 and 380 mg/kg), vehicle

and cyclophosphamide were administered by single oral gavage to groups of mice (5/sex/dose). The groups of mice treated with 1,3-dichloropropene and the vehicle were sacrificed at 24- and 48-h after treatment. Mice treated with the positive control were sacrificed 24-h after treatment. The high dose in this study was 380 mg/kg (60% of the LD_{50}), which was a toxic dose in males resulting in death of 1 out of 4 males in the 24-h group and 3 males in the 48-h group, and toxicity of the bone marrow in females. The study is in compliance with OECD TG 474 (2016) with only minor deviations relating to the number of cells scored (200 vs 500 erythrocytes scored for %PCE; 1000 vs 4000 PCE scored for MN), although reporting deficiencies (a lack of data for individual animals and no HCD) are also noted.

At the end of the specified intervals following dosing, the animals were sacrificed, and bone marrow samples were obtained, and smears were prepared on microscope slides. The ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) in the bone marrow cells was determined for each animal by examining approximately 200 erythrocytes (expressed as % PCE). For each animal, the incidence of micronucleated polychromatic erythrocytes (MNPCE) was determined in 1000 PCE. The % PCE observed in the groups treated with 1,3-dichloropropene did not suggest depressed erythropoietic activity except in females treated with 380 mg/kg and sacrificed at 48 h. The % PCE in this group was 41.4 compared to 61.5 in the appropriate negative control group. The highest increases in the frequencies of MNPCE were observed at the top dose level tested, in both males and females from the 48-h sacrifice group, however, these increases were not significant (when examined at the 1% level; $p < 0.01$) when compared to negative controls. The positive control gave a satisfactory response.

Table 49: Frequencies of Micronucleated Polychromatic Erythrocytes (MN-PCE) in Bone Marrow of Male Mice (Anonymous 26, 1985)

Treatment (mg/kg)	24-h Sacrifice				48-h Sacrifice			
	No. of mice	PCE Examined	MNPCE	% PCE	No. of mice	PCE Examined	MNPCE	% PCE
Neg. Control	5	5000	1.0	56.8	5	5000	2.1	57.3
(1,3-D) 38	5	5000	1.6	56.8	5	5000	0.6	61.5
(1,3-D) 115	5	5000	1.4	55.1	5	5000	0.6	61.5
(1,3-D) 380	4	4000	1.8	60.3	2	2000	1.5	52.0
Pos. Control								
(CP) 120	5	5000	47.0*	34.1	ND			

* Statistically different from controls p<0.01, ND – Not determined.

Table 50: Frequencies of Micronucleated Polychromatic Erythrocytes (MN-PCE) in Bone Marrow of Female Mice (Anonymous 26, 1985)

Treatment (mg/kg)	24-h Sacrifice				48-h Sacrifice			
	No. of mice	PCE Examined	MNPCE	% PCE	No. of mice	PCE Examined	MNPCE	% PCE
Neg. Control	5	5000	1.4	68.8	5	5000	0.2	61.5
(1,3-D) 38	5	5000	0.6	64.1	5	5000	0.6	64.2
(1,3-D) 115	5	5000	0.8	62.7	5	5000	0.2	63.3
(1,3-D) 380	5	5000	0.8	62.1	5	5000	1.6	41.4
Pos. Control								
(CP) 120	5	5000	45.8*	49.0	ND			

* Statistically different from controls p<0.01, ND – Not determined.

Under the conditions of this study, 1,3-D was considered negative in the mouse bone marrow micronucleus test.

There was also no evidence of increased micronucleus frequency in the study of Ghia *et al.* (1993), where 1,3-D was tested in male Sprague-Dawley rats up to 125 mg/kg in DMSO at a dose volume of 10 mL/kg. Investigation of higher dose levels was precluded due to lethality (three rats died within 24 h after the administration of 250 mg/kg 1,3-D). Hepatocytes, and erythroblasts from bone marrow and spleen were evaluated for micronucleus formation. In order to include an assessment of hepatocytes, rats were partially hepatectomised 20 hours prior to dose administration. N-nitrosodimethylamine (NDMA), used as positive

control (Vehicle not stated), induced, as expected, a clear-cut clastogenic response in the three target organs. A summary of the data is shown in Tables 51 and 52. Although a small increase in MN PCE was observed in spleen and bone marrow, this did not achieve statistical significance (Wilcoxon two-sample (two-tailed) test, p>0.05). This study deviated from the current OECD TG 474, not only by the inclusion of partial hepatectomy, but also because only one sample, 48 h post-dosing, was included for each tissue, instead of at least two recommended. There is also a lack of methodology detail reported, no information on MN frequencies in individual animals and no HCD presented.

Table 51: Frequencies of Micronucleated Polychromatic Erythrocytes in Bone Marrow and Spleen of Rats after a Single oral Administration of 1,3-D (Ghia *et al.,* 1993)

Treatment conditions	Bone Marrow			Spleen		
	No. PCEs	Frequency of PCEs (%)	Frequency of MNPCEs (%)	No. PCEs	Frequency of PCEs (%)	Frequency of MNPCEs (%)
Control	5140	60.2	0.79	5050	13.0	0.59
1,3-D 125 mg/kg	5092	52.1	1.37	5094	17.3	1.57
NDMA 10 mg/kg	3139	64.4	1.41	3032	15.2	5.95

Table 52: Frequency of Micronucleated Hepatocytes in Rats Treated with a Single oral Dose of 1,3-D (Ghia *et al.*, 1993)

Treatment conditions	# of hepatocytes observed	Frequency of micronucleated hepatocytes (%)	Frequency of binucleated hepatocytes (%)	Mitotic index (%)
Control	5083	1.58	84.0	5.9
1,3-D 125 mg/kg	5045	1.19	64.7	5.3
NDMA 10 mg/kg	3007	11.31	53.0	6.3

The potential for 1,3-D to cause DNA damage following *in vivo* exposure has been evaluated in two alkaline elution assays (Ghia *et al.,* 1993; and Kitchin and Brown, 1994) and a UDS assay (Ghia *et al.,* 1993). Ghia *et al.* (1993) reported a positive alkaline elution assay (i.e., DNA strand break) responses in rat liver, stomach, and kidney following oral gavage or i.p. administration of 62.5 and 125 mg/kg in vehicle DMSO, but not in lung, brain, or bone marrow (Table 53). In contrast to the positive alkaline elution results in liver cells, in the accompanying UDS assay, there was no evidence of UDS in hepatocytes isolated from rats treated via oral gavage with 125 mg/kg 1,3-D (Table 56). The alkaline elution assay was also used to evaluate the effect of GSH depletion and inhibition of cytochrome P450 activity on the amount of DNA fragmentation in the liver following oral administration of 1,3-D. GSH depletion was produced by an i.p. injection of either D, L-buthionine [S, R] sulfoximine (BSO) or diethyl maleate (DEM) administered prior to 1,3-D dosing, either 24 and 12 h for BSO or 1 h for DEM. P450's were inhibited by an i.p. injection of methoxsalen (MS), a suicide inhibitor of cytochrome P450, administered 30 min prior to 1,3-D. No justification for the timing of BSO, DEM or MS dosing was provided. The data showed that in rats pre-dosed with MS, but not with BSO or DEM, there was a statistically significant reduction in the degree of liver DNA fragmentation induced by 1,3-D (Table 54). Subsequent evaluation of GSH levels in rats dosed with 1,3-D (at 62.5 and 125 mg/kg) with and without pre-administration of DEM, demonstrated that 1,3-D alone caused a dose-related reduction in GSH, which peaked at 1 h after administration, that was not significantly increased by pre-administration of DEM (Table 55). It was reported that administered alone, MS, DEM and BSO did not induce any change of DNA elution rate, compared to concurrent controls given the vehicle alone, although the data are not shown in the published paper. Ghia *et al.* (1993) concluded that in liver, cytochrome P450-mediated metabolism plays a significant role in the DNA damage caused by 1,3-D.

Table 53: Summary of data from alkaline elusion assay - DNA fragmentation in some tissues of rats after treatment with 1,3-D (Ghia *et al.*, 1993)

Tissue	Dose (mg/kg)	Route of administration	Hours after treatment	No. of animals	Kt/Kc (mean ± SD)	Significance level (p)
Liver	0			12	Kc = 0.0177 ± 0.0033	
	62.5	Oral	3	4	1.28 ± 0.22	<0.02

	125	Oral	1	3	2.28 ± 0.28	<0.002
		Oral	3	6	2.83 ± 0.20	<0.002
		Oral	24	3	1.52 ± 0.25	<0.002
		i.p.	3	3	1.57 ± 0.08	<0.002
	250	Oral	3	3	4.11 ± 0.15	<0.002
Gastric Mucosa	0			7	Kc = 0.0317 ± 0.0170	
	62.5	Oral	3	3	1.09 ± 0.08	=0.016
	125	Oral	1	6	1.46 ± 0.17	=0.002
		Oral	3	3	1.79 ± 0.23	=0.016
		Oral	24	3	1.18 ± 0.23	>0.10
	250	Oral	3	6	1.99 ± 0.04	=0.016
Kidney	0			7	Kc = 0.0203 ± 0.0021	
	125	Oral	2	5	1.24 ± 0.23	=0.008
		Oral	24	4	1.10 ± 0.06	>0.10
		i.p.	3	3	1.89 ± 0.14	=0.016
Lung	0			4	Kc = 0.0214 ± 0.0017	
	125	Oral	3	3	1.19 ± 0.08	>0.10
		Oral	24	3	1.08 ± 0.08	>0.10
		i.p.	3	3	1.02 ± 0.04	>0.10
Bone Marrow	0			5	Kc = 0.0195 ± 0.0014	
	125	Oral	3	3	1.16 ± 0.11	>0.10
		Oral	24	3	1.13 ± 0.05	>0.10
		i.p.	3	3	1.07 ± 0.08	>0.10
Brain	0			4	Kc = 0.0256 ± 0.0003	
	125	Oral	3	4	1.07 ± 0.03	>0.10

Table 54: Effects of GSH depletion and cytochrome P450 activity inhibition on the amount of DNA fragmentation induced by 1,3-D in the rat liver (Ghia et al., 1993)

Treatment conditions (Oral 1,3-D administration)	No. of rats	Relative DNA elution rate: Kt/Kc (mean ± SD)
1,3-D 62.5 mg/kg	4	1.28 ± 0.22
1,3-D 125 mg/kg	6	2.83 ± 0.20
1,3-D 62.5 mg/kg + DEM	4	1.63 ± 0.46
1,3-D 125 mg/kg + DEM	4	3.22 ± 0.09
1,3-D 125 mg/kg + BSO	4	2.74 ± 0.18
1,3-D 125 mg/kg + MS	4	1.86 ± 0.12*

Kc = 0.0177 ± 0.0033 (see table above)
* p = 0.01 versus the same dose of 1,3-D alone.

Table 55: GSH depletion induced in the rat liver by 1.3-D and 1,3-D plus DEM (Ghia *et al.*, 1993)

	% GSH variation from controls (mean ± SD)		
Treatment Conditions	**1 hr**	**3 hr**	**24 hr**
1,3-D 62.5 mg/kg	-44 ± 5	-31 ± 8	+2 ± 12
1,3-D 125 mg/kg	-84 ± 7	-72 ± 11	+7 ± 16
DEM + 1,3-D 62.5 mg/kg	-90 ± 4	-54 ± 13[a]	+5 ± 15[a]
DEM + 1,3-D 125 mg/kg	-95[b]	-87[b]	+6[b]

[a] Mean based on four surviving rats.
b Mean based on two surviving rats

Table 56: UDS in hepatocytes of rats treated with a single dose of 1,3-D (125 mg/kg) (Ghia *et al.*, 1993)

Treatment conditions	No. of rats	Nuclear grain count (mean ± SD)	Cytoplasmic grain count (mean ± SD)	Net nuclear grains (mean ± SD)	% Repair (mean ± SD)
Control	3	2.5 ± 1.8	2.3 ± 1.8	0.2 ± 2.0	1.7 ± 2.0
1,3-D	4	4.6 ± 2.7	4.1 ± 2.6	0.5 ± 2.5	6.2 ± 6.6
NDMA	3	24.3 ± 15.0	2.2 ± 2.0	22.1 ± 12.4	92.8 ± 11.4

Kitchin and Brown (1994) reported DNA breakage by alkaline elution in hepatocytes of rats. Very little about the methodology and doses of 1,3-D tested was reported. The study was conducted using female Sprague-Dawley rats (CD strain). 1,3-dichloropropene was obtained from the NTP repository. The vehicle was corn

oil. Groups of 7-9 rats received six doses in a range of 3000-fold difference. The initial dose tested was 94 mg/kg. Doses were administered by gavage twice, the first, 21-h before sacrifice, and the second, 4 h before sacrifice. The rat hepatic DNA damage assay (alkaline elution) was performed as previously described (Kitchin and Brown, 1989). The data were analysed by analysis of variance, and where statistically significant differences were found, they were then evaluated with a Student's t-test. All comparisons were made between treatment groups and concurrent vehicle treated rats. If day effects of measurement were present, data were converted to percent of concurrent control values. A value of $P< 0.05$ was considered a statistically significant increase. Regression analyses were carried out using the SAS System Release 6.06.

The lowest DNA-damaging dose (LOEL) was 94 mg/kg while the highest dose of 1,3-dichloropropene that did not cause rat hepatic DNA damage was 9.4 mg/kg (NOEL). To place the observation of DNA damage in a biological perspective, the dose-response curve was normalized with the oral rat LD50 value. Accordingly, 1,3-dichloropropene damaged DNA at doses of ≈ 40% of the LD50 or higher. Regression analysis showed good fit of the dose-response data to linear model and even better to quadratic model. Under the conditions of this study, it was concluded that 1,3-dichloropropene caused DNA damage in rat liver.

In the ninth study, Anonymous 145 (2023), the potential of 1, 3-dichloropropene to induce micronuclei in young erythrocytes (i.e., reticulocytes) in mouse peripheral blood was evaluated following 2 consecutive days of treatment by oral gavage. Three doses at 125, 250 and 500 mg/kg/day were evaluated for both males and females in the range-finding phase, with doses of 81.25, 162.5 and 325 mg/kg/day (males) and 87.5, 175 and 350 mg/kg/day (females) in the definitive phase. In both phases, animals were dosed once daily for 2 consecutive days via oral gavage, except in the 500 mg/kg/day group males and females in the range-finding phase, which were all found dead or euthanized in extremis on Day 2 prior to dose administration.

The following parameters and end points were evaluated in this study: mortality, clinical signs, body weights, body weight gains, body temperature, and micronucleus evaluation (definitive phase only).

Range-Finding Phase

Test substance-related mortality was noted in the 500 mg/kg/day group males and females. On Day 2 (prior to Day 2 dosing), all 500 mg/kg/day group males and 2/3 females were found dead, and the remaining female was euthanized in extremis. Clinical observations were only noted in the 500 mg/kg/day group females and included decreased activity, labored and shallow breathing, cold to touch, suspected dehydration (moderate), and weak. A body weight loss of 5.6% was noted from Day 1 to 2 for the female euthanized in extremis. In addition, decreased body temperature was noted in all 500 mg/kg/day group males and females at the 1- to 4-hour postdosing collections on Day 1. There were no abnormal macroscopic findings noted at necropsy for the animals found dead or euthanized in extremis.

All remaining animals survived to the scheduled euthanasia. There were no test substance-related clinical observations or effects on body weight for the animals that survived to the scheduled euthanasia.

Definitive Phase

Test substance-related morality was noted in the 325 mg/kg/day group males and 350 mg/kg/day group females. One 325 mg/kg/day group male was euthanized in extremis on Day 2 (prior to dosing), and another male was found dead at approximately 5.5 hours post the Day 2 dose. Clinical observations in these males included decreased activity, shallow breathing, cold to touch, eye(s) closed (partially to completely), erected fur, hunched posture, and/or lying on side. From Day 1 to 2, these males lost 8.0% and 8.3% of body weight. The last recorded body temperature for these males was 24.9°C (Day, 2 predosing collection) and 25.7°C (Day 2, five-hour postdosing collection), which were 13.1°C and 14.4°C lower, respectively, than the Day 1 predosing temperature. There were no abnormal macroscopic findings noted at necropsy. On Day 3, one 350 mg/kg/day group female was euthanized in extremis and clinical observations included decreased activity, cold to touch, eyes closed (partially), erected fur, and hunched posture. From Day 1 to 3, this female lost 6.4% of body weight. A last recorded body temperature was 27.7°C (Day 2, five-hour postdosing collection), which was 10.1°C lower than the Day 1 predosing temperature. There were no abnormal macroscopic findings noted at necropsy. A single female in the 175 mg/kg/day group was euthanized in extremis on Day 2 (prior to dosing); however, this death was not considered test substance-related but instead, due to limited usage and a laceration of the left hind leg.

All remaining animals survived to the scheduled euthanasia. There were no test substance-related clinical observations for the animals that survived to the scheduled euthanasia.

The analyzed dosing formulations contained 85.4% to 112% of the test substance which was within the protocol-specified range of target concentrations for suspensions (85% to 115%), were homogeneous, and were stable for up to 9 days when stored at -80°C, with the following exceptions. The 22 Aug 2022 formulations for the 81.25 mg/kg/day group males, 325 mg/kg/day group males, and 350 mg/kg/day group females (16.25, 65, and 70 mg/mL groups, respectively) were flagged for quality failure and the data were rejected. Backup sample results were 96.8%, 96.6%, and 112% of target with an RSD of 8.60%, 4.77%, and 8.91%, respectively. This did not negatively impact the quality or integrity of the data or the outcome of the study the backup samples met the protocol-specified acceptance criteria.

Test substance-related lower mean body weight gains were noted in the 325 mg/kg/day group males throughout the dosing period and from Day 2 to 4 in the 350 mg/kg/day group females.

Test substance-related decreased mean body temperatures were noted in the 325 mg/kg/day group males and 350 mg/kg/day group females on Days 1 and 2 at the 2- and 5-hour postdosing collections. On Day 2, mean body temperatures in the 325 mg/kg/day group males and 350 mg/kg/day group females were 35.04°C and 37.59°C at the predose collection, 34.45°C and 34.43°C at the 2-hour postdosing collection, and 33.60°C and 33.34°C at the 5-hour postdosing collection, respectively.

There was a decreasing non-monotonic trend in the percentage of reticulocytes (%RET) for males and females, and an increasing non-monotonic trend in percentage of micronucleated reticulocytes (%MN-RET) for females. The decreasing trend in %RET suggested proof of 1,3-dichloropropene exposure to the hematopoietic system in both males and females, consistent with previous data indicating systemic availability of the test substance after oral administration. In the 0, 81.25, 162.5, and 325 mg/kg/day groups mean %MN RET for males were 0.20%, 0.21%, 0.21%, and 0.24%, respectively. In the 0, 87.5, 175, and 350 mg/kg/day groups mean %MN-RET for females were 0.15%, 0.20%, 0.19%, and 0.25%, respectively. These data indicate that 1,3-dichloropropene was negative for induction of micronuclei in young peripheral blood erythrocytes (i.e., reticulocytes) at dose levels up to 325 mg/kg/day for males and 350 mg/kg/day for females under the conditions of this assay.

In conclusion, administration of 1,3-dichloropropene by once daily oral gavage to Crl:CD1(ICR) mice at dose levels of 81.25, 162.5, and 325 mg/kg/day to males and 87.5, 175, and 350 mg/kg/day to females for 2 days resulted in lethality at the highest dose levels and a negative response for induction of peripheral blood micronuclei at all dose levels.

Table 57: Summary of Micronucleus Data: Males (Anonymous 145, 2023)

Treatment	Animal Number	No. Ret	No. MN-RET	No. NCE	No. MN-NCE	RET(%)	MN-RET(%)
Vehicle control	4001	4938	14	325659	566	1.50	0.28
	4002	4934	16	228838	385	2.11	0.32
	4003	4945	8	398103	557	1.23	0.16
	4004	4936	8	326004	556	1.49	0.16
	4005	4950	4	373901	443	1.31	0.08
Mean ± SD						1.53 ± 0.35	0.20 ± 0.10
1,3-D 81.25 mg/kg/day	5001	4941	12	372453	358	1.31	0.24
	5002	4925	10	251083	419	1.92	0.20
	5003	4942	7	374617	350	1.30	0.14
	5004	4927	15	254878	460	1.90	0.30
	5005	4952	7	323441	322	1.51	0.14
Mean ± SD						1.59 ± 0.31	0.21 ± 0.07
1,3-D 162.5 mg/kg/day	6001	4932	11	344757	544	1.41	0.22
	6002	4950	13	313275	453	1.56	0.26
	6003	4928	6	139907	168	3.40	0.12
	6004	4937	11	235064	264	2.06	0.22
	6005	4951	10	360570	370	1.36	0.20
Mean ± SD						1.96 ± 0.85	0.21 ± 0.05
1,3-D 325 mg/kg/day	7001	4917	8	600488	612	0.81	0.16
	7003	4896	15	442191	677	1.10	0.31
	7005	4897	12	377151	465	1.28	0.24

	7006	4916	11	487176	729	1.00	0.22
	7007	4930	14	425350	460	1.15	0.28
Mean ± SD						1.07 ± 0.18[a]	0.24 ± 0.06
Positive control	11001	4894	45	838127	810	0.59	0.91
	11002	4898	31	891320	1026	0.55	0.63
	11003	4870	49	1321454	1726	0.37	1.00
Mean ± SD						0.50 ± 0.12[b]	0.85 ± 0.19[b]

No. = number; RET = reticulocyte; MN = micronucleated; NCE = normochromatic erythrocyte; SD = standard deviation, E = Excluded from interpretation as < 4,000 RET were analyzed. Vehicle Control = Corn oil. Positive Control = Cyclophosphamide 25 mg/kg/day. [a] Statistically significant dose-related trend ($p \leq 0.05$, Cochran-Armitage); [b] Statistically significant when compared to the corresponding vehicle control cohort ($p \leq 0.05$, Dunnett's).

Table 58: Summary of Micronucleus Data: Females (Anonymous 145, 2023)

Treatment	Animal Number	No. Ret	No. MN-RET	No. NCE	No. MN-NCE	RET(%)	MN-RET(%)
Vehicle control	4501	4951	5	567843	832	0.86	0.10
	4502	4918	7	543587	652	0.90	0.14
	4503	4913	4	274809	327	1.76	0.08
	4504	4942	6	433958	595	1.13	0.12
	4505	4932	15	484663	895	1.01	0.30
Mean ± SD						1.13 ± 0.36	0.15 ± 0.09
1,3-D 87.5 mg/kg/day	8501	4932	9	223893	231	2.16	0.18
	8502	4926	11	348769	491	1.39	0.22
	8503	4952	13	459008	522	1.07	0.26
	8504	4922	6	353541	303	1.37	0.12
	8505	4930	10	471614	546	1.04	0.20
Mean ± SD						1.41 ± 0.45	0.20 ± 0.05
1,3-D 175 mg/kg/day	9502	4932	10	323755	473	1.50	0.20
	9503	4928	11	291778	242	1.66	0.22
	9504	4939	6	677009	738	0.72	0.12
	9505	4922	13	373634	348	1.30	0.26
	9506	4952	8	589343	696	0.83	0.16
Mean ± SD						1.20 ± 0.41	0.19 ± 0.05
1,3-D 350 mg/kg/day	10501	4912	6	600544	770	0.81	0.12
	10502	4896	15	450250	770	1.08	0.31
	10504	4843	20	900063	1508	0.54	0.41
	10505	4925	8	291267	232	1.66	0.16
	10506	4884	13	709451	814	0.68	0.27
Mean ± SD						0.95 ± 0.44[a]	0.25 ± 0.12[a]
Positive control	11501	4876	50	1128509	1696	0.43	1.02
	11502	4854	73	781835	1952	0.62	1.48
	11503	4879	49	558580	787	0.87	0.99
Mean ± SD						0.64 ± 0.22	1.16 ± 0.28[b]

No. = number; RET = reticulocyte; MN = micronucleated; NCE = normochromatic erythrocyte; SD = standard deviation, E = Excluded from interpretation as < 4,000 RET were analyzed. Vehicle Control = Corn oil. Positive Control = Cyclophosphamide 25 mg/kg/day. [a] Statistically significant dose-related trend ($p \leq 0.05$, Cochran-Armitage); [b] Statistically significant when compared to the corresponding vehicle control cohort ($p \leq 0.05$, Dunnett's).

Summary of *in vivo* somatic cell data

Two transgenic rodent gene mutation assays (Anonymous 25, 1997 and Anonymous 24, 2018) have been conducted with 1,3-D, and the most recent assay (Anonymous 24, 2018) is fully compliant with OECD TG 488 (2013) and was conducted to GLP. Both studies demonstrate that 1,3-D does not cause gene mutations in the liver, kidneys and lungs of transgenic rodents, therefore it is concluded that the mutagenicity induced by 1,3-D in bacteria is not expressed *in vivo*.

Six bone marrow micronucleus tests (5 in mice and 1 in rats) have been conducted with 1,3-D (Anonymous 26, 1985; Shelby *et al.*, 1993; Kevekordes *et al.*, 1996; Anonymous 47, 1999; Anonymous 145, 2023 and Ghia *et al.*, 1993, respectively). Only Anonymous 145 (2023) is fully compliant with OECD TG 474 (2016), with the majority of deviations in the remaining studies relating to the characterisation of the test substance used, a deficiency in the number of PCE scored for MN and a lack of reported information for individual animal MN PCE frequencies and HCD, or inappropriate route of administration. Four of the studies reported negative

results for 1,3-D (Anonymous 26, 1985; Anonymous 47, 1999; Ghia *et al.*, 1993; Anonymous 145, 2023). In Anonymous 47, a bone marrow micronucleus test via an inhalation route with trans 1,3-D dosing up to 600 ppm (535 ppm after correction) in groups of five male mice at two sample times, 24 and 48 hours after dosing, MN were evaluated in 2000 polychromatic erythrocyte (PCE) cells. Positive (mitomycin C via single i.p. dose) and negative controls gave adequate responses for a valid study. All doses of trans 1,3-D had %MNPCE frequencies that were similar to and not statistically different from concurrent vehicle controls. For Anonymous 26 and Ghia *et al.*, 1993, it is noted that in both cases increases in MNPCE were apparent, which failed to achieve the level of statistical significance defined by the authors as being indicative of a positive response. Two of the studies reported positive results (Shelby *et al.*, 1993; Kevekordes *et al.*, 1996). However, the study of Kevekordes *et al.*, (1996) is considered unacceptable for consideration due to significant deviations from OECD 474 (2016), most notably the use of just a single animal/sex for the negative control group, questions over the health status of the animals used, and a lack of any explanation for the clear difference in responses between male and female animals. The study of Shelby *et al.* (1993), reported increases in MNPCE frequencies only after a single administration at 150 mg/kg and only when sampled 48 h after dosing. Negative results were obtained at the 24 h sample time and at all dose levels tested using a 3-dose, 24 h sample regimen. Furthermore, the increased MNPCE frequency was shown to be within the laboratory's HCD and is therefore considered to be equivocal according to modern evaluation criteria (OECD TG 474, 2016). Anonymous 145 evaluated micronuclei in the peripheral blood of 1,3-D treated mice in a range finder and one main study in a GLP and OECD 487 complaint study. Groups of five male and female mice were dosed over 2 consecutive days of treatment by oral gavage, sampling 48 hours after the first dose.. In the range finder 500 mg/kg/day resulted in lethality and as such the MTD was determined as 325 mg/kg in males and 350 mg/kg in female mice. In the main study phase, micronucleated reticulocytes (MNRET) were scored in approximately 5000 reticulocytes via flow cytometry. Numbers of normochromatic erythrocytes (NCE's) were also scored. There were no adverse clinical signs in the main study and all 1, 3-D dose groups from both sexes resulted in %MNRET that were not statistically different to the concurrent control group and all values were within the 95th percentile of the historical control range, positive controls (single i.p. dose of MMC). As such the study concluded that 1,3-D was negative for induction of micronuclei in the peripheral blood of mice.

Three studies (in two reports, Ghia *et al.*, 1993; Kitchin and Brown, 1994) are available in rats that report the DNA damage effects of 1,3-D. These data (2 alkaline elution assays and an UDS assay) are not apical endpoints of mutagenicity, but rather are indicators of genotoxicity. Ghia *et al.* (1993) found 1,3-D negative in a single time point (2-4 h) liver UDS assay and in an alkaline elution assay 1,3-D did not induce DNA damage in lung, brain, or bone marrow; however, positive results for DNA damage were observed in liver, stomach, and kidney. Kitchin and Brown (1994) also reported positive DNA damage in rat liver in the alkaline elution assay. Relevant supporting information is also available in Ghia *et al.* (1993) that provides further weight of evidence for the likely mechanism behind observed 1,3-D genotoxicity. Cytochrome P450-mediated metabolism has been shown to play a significant role in the DNA damage caused by 1,3-D. Furthermore, 1,3-D has been shown to cause significant GSH depletion within 1 hour of administration of a single 125 mg/kg dose. The observations of 1,3-D-induced GSH depletion *in vivo* provide further evidence that 1,3-D-mediated genotoxicity is directly linked to the role of glutathione in protecting cells from this toxicity.

Genotoxicity in germ cells

In the sex-linked recessive lethal (SLRL) test in *Drosophila melanogaster*, followed by the test for induction of reciprocal translocations (RT) (Valencia *et al.*, 1985), the frequency of SLRL caused by 1,3-dichloropropene was 0.3% (for the three broods combined) and exceeded 0.2% over the control frequency (0.12%). Generally, a test was considered positive if the frequency of lethals in the treated series exceeded 0.2% over the control frequency; therefore, although the statistical significance of this increase was not reported, the test compound could be considered positive. The examination of individual broods showed that the effect was similar when compared to its appropriate control value. Nevertheless, according to the table below when all three broods were summed, 1,3-dichloropropene induced SLRL in only one trial indicating that the positive response was not reproducible; thus it should be considered a weak positive at most.

Table 59: Results for the 1,3-D sex-linked recessive lethal (SLRL) test in Drosophila melanogaster (Valencia *et al.*, 1985)

	Mortality [a]	Sterility [b]	Lethals: brood			Tests: brood			Total lethals	Total tests	Lethals (%)
Treated			**1**	**2**	**3**	**1**	**2**	**3**			
Trial I			7	2	5	1343	1088	1106	12	2385	0.43
Trial II			3	3	0	1373	1088	8238	6	2399	0.18
Total	33	10	10	5	5	2716	1924	1944	20	6584	0.30
Control			**1**	**2**	**3**	**1**	**2**	**3**			
Trial I			3	1	0	1200	1200	1171	4	3571	0.11
Trial II			3	0	1	1159	1119	1069	4	3347	0.12
Total			6	1	1	2359	2319	2240	8	6918	0.12

For the RT test, the males were mated (10 males and 20 females per vial) to virgin females. In order to age the treated sperm, fertilized females were kept on regular culture medium and transferred every 3 or 4 days for as long as they produced eggs. The RT data were compared to the combined historical control for three laboratories (data not reported), and the conditional binomial test was used to determine the significance (at the 0.5 level). In general, at least two translocations were required in the treated series to establish significance and a positive result; however, 1,3-dichloropropene did not induce reciprocal translocations in Drosophila melanogaster. While there were some deviations from the OECD TG 477, as highlighted (no details on methodology; the age of insects, and the number of males treated; F2 cultures established and F2 cultures without progeny were not reported; statistical significance of the increase in the frequency of SLRL caused by 1,3-dichloropropene not reported.), it should be noted that this guideline was deleted in 2014 as the test method had fallen out of favour due to other test methods that provide greater reproducibility and are conducted in mammalian systems (*in vivo* and *in vitro*).

In the dominant lethal assay (Anonymous 27, 1997), groups (30/group) of male Sprague Dawley derived rats were exposed to 1,3-D vapours by inhalation at targeted concentrations of 0 (negative control), 10, 60 and 150 ppm for 10 weeks (6 h/day, 7 days/week). The positive control was a group of 30 males that were given a single dose of Cyclophosphamide monohydrate (CP) 48 h prior to the start of the matings. At the end of exposure period, each male was co-housed with untreated virgin females for 2 consecutive mating trials (1 week/trial, 2 females/male). Females were euthanized and necropsied 13 days after the conclusion of each weekly mating trial. The uterine contents were examined, and the following data recorded: a) the number of corpora lutea; b) the number and position of implants *in utero*; c) the number of live implants; and d) the number and position of resorption sites. Based on the observations the following indices were calculated: Male fertility index (%) and Pregnancy Rate (%). For each male, the following indices were also calculated: Pre-implantation loss (%) and Post-implantation loss (%). In both mating trials, the fertility of males as well as the pregnancy rate of females co-housed with the treated males was not significantly affected in any treatment group. CP treatment induced a significant increase in post-implantation loss during both mating periods. Under the conditions of this study, 1,3-dichloropropene did not induce dominant lethal mutations in the germ cells of male CD rats at exposure levels up to 150 ppm.

Summary of *in vivo* germ cell data

Two studies providing information on effects in germ cells are available. The only GLP, guideline compliant study (OECD TG 478; Anonymous 27, 1997) demonstrated that 1,3-D did not induce dominant lethal mutations in male rats. Although Valencia *et al.*1985 reported 1,3-D as positive in a SLRL test in *Drosophila melanogaster*, the effect was weak and only apparent in 1/2 of the trials conducted. Furthermore, OECD TG 477 has been deleted due to its insensitivity and lack relevance to mammalian biology. It is therefore concluded that 1,3-D does not cause genotoxicity in the germ cells of male rats.

Human information

There are no reports of genotoxicity studies in humans.

Other relevant information

The major pathway for detoxification of 1,3-D has been shown to be via glutathione conjugation. A minor pathway is via hydrolysis with a third trace pathway being identified as oxidation resulting in the formation of reactive 1,3-D epoxide. This trace pathway is considered to occur only at high (approx. LD50) doses (Bartels *et al.*, 2004).

Summary and discussion of mutagenicity

Based on an evaluation of the available data it can be concluded that there is evidence 1,3-D causes gene mutations in bacteria, in tester strains *S. typhimurium* TA100 and TA1535 in the presence and absence of exogenous metabolic activation. Metabolic activation does not increase the mutagenic response, and co-incubation with glutathione prevents a mutagenic response in the assay. There is no evidence that 1,3-D causes gene mutations *in vitro* in mammalian cells. Furthermore, there is no evidence that 1,3-D induces genetic mutation *in vivo*, based on the results of transgenic rodent assays in Big Blue® transgenic rats and mice (somatic cells).

Based on the available data there is some evidence that 1,3-D has clastogenic potential in an *in vitro* chromosome aberration assay (Anonymous 38, 1988); however, the extent to which cytotoxicity, pH and/or osmolarity changes may have confounded the results of this assay are unclear. In *vivo*, data are mixed, the more recent OECD guideline compliant studies and those treated via oral gavage or inhalation routes are generally negative or have increases that are within historical ranges and not well reproduced between studies. Treatment via the i.p. route is more often difficult to interpret or equivocal, this route of administration is no longer recommended. Of the six studies available, the Kevekordes *et al.* (1996) study is considered unacceptable for evaluation due to significant deviations from OECD TG 474 (2016), including a lack of characterisation of the test material used and queries over the health status of the animals used. Of the remaining studies, three also have some deficiencies with respect to OECD TG 474 (mostly due to a reduced number of cells analysed and a lack of reported details for individual animals and HCD). Shelby *et al.*, 1993, reports clearly negative results in male mice administered 1,3-D over 3 days and sampled 24 h after the 3rd administration. However, when administered once an equivocal increase in MNPCE (statistically significant but clearly within HCD) was apparent at the 48 h but not at the 24 h timepoint. Evaluation of these data using modern OECD TG 474 (2016) criteria would suggest an equivocal conclusion is appropriate. Anonymous 26 (1985) and Ghia *et al.* (1993) conclude that 1,3-D does not induce MNPCE in mice and rats respectively, due to a lack of statistical significance according to the criteria defined by the authors. The two most recent studies Anonymous 47 (1999) and Anonymous 145 (2023), a bone marrow micronucleus test via an inhalation route with trans 1,3-D and a peripheral blood micronucleus test via the oral route with 1,3-D respectively were both clearly negative. Both tests were robust and the latter fully complaint with GLP and OECD 474. Overall when tested in robust, guideline compliant tests using appropriate routes of exposure, 1,3-D is negative for the induction of clastogenicity *in vivo*.

Some indicator (i.e. non-apical endpoint) assays provide further, supplementary genotoxicity data for 1,3-D. 1,3-D was negative in an UDS assay, performed solely at the short (2-4 h) sample time. Although, *in vivo* studies provide evidence for DNA damage in the liver, stomach and kidney following oral administration of 1,3-D to rats in two separate alkaline elution assays, 1,3-D did not bind calf thymus DNA *in vitro*, either in the absence or presence of potential activating enzymes, nor in the presence of physiologically relevant glutathione concentrations.

Data from several studies indicate that 1,3-D genotoxicity is associated with depletion of glutathione (GSH), thus providing a plausible, non-specific DNA damage mechanism for the reported positive genotoxicity data. The bacterial mutagenicity study conducted by Anonymous 35 (1999; GLP and OECD TG 471 compliant), demonstrated that 1,3-D was mutagenic in bacterial strains TA100 and TA1535 both with and without exogenous metabolic activation with rat liver S9. The same laboratory performed a follow-up assessment aimed specifically at examining the effects of different metabolic activation conditions on 1,3-D mutagenicity Anonymous 31 (2004). Strain TA1535 was examined as this provided the clearest mutagenic response in the previous study (Anonymous 35, 1999). 1,3-D induced clear positive results in TA1535 in the absence and presence of purified microsomes (either rat, mouse or human). However, this response was eliminated when TA1535 was preincubated with 1,3-D in the presence of either S100 (cytosol; rat, mouse or human) + GSH or microsomes (rat, mouse or human), S100 (rat, mouse or human) + GSH.

In the alkaline elution study of Ghia *et al.* (1993), GSH levels in rats dosed with 1,3-D (at 62.5 and 125 mg/kg, i.e. dose levels similar to those shown to induce weak/equivocal increases in MN PCE in bone marrow)

decreased in a dose-related manner, peaking at 1 h after administration. Furthermore, the degree of GSH depletion was not significantly increased by pre-administration of DEM (proven to also deplete GSH).

The proposed metabolic pathway of 1,3-D in rats and mice, together with the observations that dosing of rats with 1,3-D causes GSH depletion and the evidence that mutagenic effects observed in the bacterial reverse mutation assay can be eliminated by co-incubation with GSH, provide a plausible mechanism for 1,3-D genotoxicity. The major route of 1,3-D detoxification by liver enzymes is via GSH-conjugation, however a trace pathway involving oxidation and subsequent formation of reactive 1,3-D-epoxide has been identified. At high doses of 1,3-D, i.e. those approaching the LD_{50}, GSH depletion results in an increase in the formation of the reactive 1,3-epoxide, which in turn is likely to be responsible for the observed genotoxicity.

Available genotoxicity studies conducted using cis 1,3-D (cis isomer) and trans 1,3-D (trans isomer) provide comparable findings to the corresponding studies conducted using 1,3-D (mix of isomers).

10.8.2 Comparison with the CLP criteria

According to CLP criteria (Regulation (EC) No. 1272/2008) the hazard categories for germ cell mutagens are as follows:

Table 60: Hazard categories for germ cell mutagens

Category	Criteria	Evidence
1		
1A	Substances known to induce heritable mutations in the germ cells of humans	Positive evidence from human epidemiological studies
1B	Substances regarded as if they induce heritable mutations in the germ cells of humans.	One of the following: • positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals • 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 o Derive supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or o Demonstrate the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells • positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny o e.g. an increase in the frequency of aneuploidy in sperm cells of exposed people.
2	Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans	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.

Although *in vitro* data demonstrate 1,3-D can cause gene mutations, albeit limited to bacterial systems, which lack GSH found in the *in vitro* test media of mammalian cell studies, 1,3-D has been demonstrated to be clearly negative in an *in vivo* transgenic rodent gene mutation test in somatic cells. 1,3-D is also negative for clastogenicity in OECD compliant *in vivo* micronucleus studies. Older studies showed scattered increases in MN; however, these studies all had significant deficiencies and were not compliant with the most recent OECD

guideline. Weak/equivocal evidence that 1,3-D induces structural and/or numerical chromosome damage in rodent bone marrow (micronucleus test data) is rendered non relevant in light of more recent guideline compliant studies. There was evidence of genotoxicity in supporting indictor studies (DNA damage as measured by alkaline elution) in rat liver. These findings are, however, only seen at high doses that approach the LD_{50}. At high exposures the major detoxification pathway for 1,3-D (i.e. glutathione conjugation) is overwhelmed and glutathione reserves are depleted. Under such conditions it is likely that the trace pathway of oxidation occurs resulting in an increase in reactive 1,3-D-epoxide. Under normal physiological conditions (such as those used in the *in vivo* transgenic rodent gene mutation test in somatic cells), the trace pathway results in little to no generation of reactive 1,3-D-epoxide and therefore no genotoxic effects. Negative results in a rodent dominant lethal assay, provide further, direct evidence that 1,3-D is not a germ cell mutagen.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on the available studies 1,3-D (mix of isomers), (Z)-1,3-D (cis-isomer) and (E)-1,3-D (trans-isomer) are not mutagenic and do not require any classification.

10.9 Carcinogenicity

Table 61: Summary table of animal studies on carcinogenicity

Method	Results	Remarks	Reference
Rat studies			
1) A CHRONIC TOXICITY AND ONCOGENICITY STUDY WITH DD-92 IN THE RAT VIA ORAL GAVAGE ADMINISTRATION 94.8% purity The percentage of isomers not available. DD-92 was previously a tradename for 1,3-D; No information on isomer composition is available in the study report. Sprague Dawley CR® rats 0, 2, 10 and 25 mg/kg bw/day OECD TG 453 GLP	NOAEL: Chronic 2 mg/kg/day Oncogenic 25 mg/kg bw/day LOAEL: Chronic: 10 mg/kg/day Chronic NOAEL: increased incidence and/or severity of hyperplasia and hyperkeratosis of the stratified squamous epithelium lining the forestomach. **Not carcinogenic**	This study is acceptable with some reservations. Deviations with respect to OECD TG 453: the group sacrificed at 12 months should have contained 20 animals/sex/group instead of the 10 used in this study. For haematology, 20 animals/sex/group should have been examined instead of 10; in addition, an analysis at three months should have been performed. The same is valid for urinalysis.	Anonymous 53, 1998
2) TELONE II SOIL FUMIGANT: **TWO-YEAR** CHRONIC TOXICITY /ONCOGENICITY STUDY IN FISCHER 344 RATS Dietary (microencapsulated) 0, 2.5, 12.5 and 25 mg/ kg/ day OECD TG 453 GLP Purity 96.0% 50.7% cis 45.1% trans	NOAEL: Chronic: 2.5 mg/kg/day Oncogenic: 2.5 mg/kg/day LOAEL: Chronic: 12.5 mg/kg/day Oncogenic: 12.5 mg/kg/day Chronic LOAEL: Depression of b.w/b.w.g. in male, day 92 onwards Foci of altered cells in the liver. Basal cell hyperplasia of the non-glandular mucosa of the stomach. Carcinogenic LOAEL:	This study is acceptable with some reservations.	Anonymous 54, 1995

	Increase incidence of benign liver tumours (hepatocellular adenomas)	Deviations with respect to OECD TG 453: the group sacrificed at 12 months should have contained 20 animals/sex/group instead of the 10 used in this study. For haematology, 20 animals/sex/group should have been examined instead of 10; in addition, an analysis at three months should have been performed. An urinalysis at three months should have been performed.	
3) TELONE II SOIL FUMIGANT: **2-YEAR INHALATION** CHRONIC TOXICITY-ONCOGENICITY STUDY IN RATS **-- INTERIM REPORT:** 6- AND 12-MONTH INTERIM SACRIFICE OF RATS 0, 5, 20 or 60 ppm (0, 4.43, 17.74, 53.22 mg/kg/d) Purity 92.1% 49.5% cis 42.6% trans Fischer 344 albino rats OECD TG 453 GLP	NOAEL: Chronic: 20 ppm (17.74) mg/kg/day) LOAEL: Chronic: 60 ppm (53.22mg/kg/day) Slight depression of in-life body weights of both sexes	This study is acceptable with some reservations. Deviations with respect to OECD TG 453: the groups sacrificed at 6 and 12 months (interim report) should have contained 20 animals/sex/group instead of the 10 used in this study. Haematology and urinalysis at three months should have been performed. Ophthalmological examinations not conducted. Food consumption not conducted	Anonymous 55, 1985
4) TELONE* II SOIL FUMIGANT: **2-YEAR INHALATION** CHRONIC TOXICITY-ONCOGENICITY STUDY IN RATS 0, 5, 20 and 60 ppm. (0, 4.86, 19.44, 58.32 mg/kg/d) Purity 92.1% 49.5% cis 42.6% trans Fischer 344 albino rats OECD TG 453 GLP	NOAEC: Chronic: 20 ppm (19.44 mg/kg/day) LOAEC: Chronic: 60 ppm (58.32mg/kg/day) Depression of body weights and Microscopic changes of nasal epithelium. (Decreased thickness olfactory epithelium, Erosions of olfactory epithelium, Submucosal Fibrosis) **Not carcinogenic.**	This study is acceptable with some reservations. Deviations with respect to OECD TG 453: Haematology and urinalysis at three months should have been performed. Ophthalmological examinations not conducted. Food consumption not conducted. Note: results from the animals sacrificed after 6 and 12 months of exposure level have been reported previously (Anonymous 55, 1985)	Anonymous 147, 1985

5) National Toxicology Program (NTP). (1985) Toxicology and carcinogenesis studies of Telone II® (technical-grade 1,3-dichloropropene containing 88%–90% 1,3-dichloropropene, 2.5% 1,2-dichloropropane, 1.5% trichloropropene isomer, and 1% epichlorohydrin as a stabilizer) in F344/N rats and B6C3F1 mice (gavage studies). U.S. Department of Health and Human Services Technical Report Series No. 269 **F344 rats – 0, 25 and 50 mg/kg** **OECD Guideline – TG 451** **GLP - yes**	On the basis of forestomach and liver neoplasms in rats, the LOAEL for cancer in the study is 21.4 mg/kg (50 mg/kg/day × 3 days/7 days) and the NOAEL for rats is 10.7 mg/kg (25 mg/kg/day × 3 days/7 days)	Not acceptable Deviations with respect to OECD TG 451 – dosing 3 times in a week and epichlorohydrin – a stabilizer that was used in Telone II may be partially responsible for the hyperplasia and squamous cell papilloma/carcinoma in the rat forestomach	In: TOXICOLOGICAL REVIEW OF 1,3-DICHLOROPROPENE (CAS No. 542-75-6) - In Support of Summary Information on the Integrated Risk Information System (IRIS), *May 2000* - EPA/635/R-00/001 Draft Assessment Report, Spain, 2018
Mouse studies			
6) Oncogenicity Study with DD-92 in the Mouse via **Oral** Gavage Administration Albino mouse **CD®-1** 0, 2, 10 and 25 mg/kg/day OECD TG 451 GLP DD-92 was previously a tradename for 1,3-D; No information on isomer composition is available in the study report.	NOAEL: Chronic: 10 mg/kg/day Oncogenic: 10 mg/kg/day LOAEL: Chronic: 25 mg/kg/day Oncogenic: 25 mg/kg/day Chronic LOAEL: Cell hyperplasia, hypertrophy in the urinary bladder. Carcinogenic LOAEL: **Benign submucosal mesenchymal tumours in the urinary bladder**	Acceptable Deviations with respect to OECD TG 451: Hematology should have been performed for all animals instead of 10 used in this study	Anonymous 52, 1997
7) Telone® Soil fumigant: two-year **dietary** chronic toxicity/oncogenicity study in **B6C3F1** Mice. (microencapsulated) 0, 2.5, 25 and 50 mg/kg/day OECD TG 453 GLP Purity 95.8% 50.7% cis 45.1% trans	NOAEL: Chronic 2.5 mg/kg/day Oncogenic NOAEL 50 mg/kg/bw/d LOAEL: Chronic 25 mg/kg/day Chronic LOAEL: Depression of in-life bw /bw gain and food consumption. **No carcinogenic**	This study is acceptable with some reservations. Deviations with respect to OECD Guideline No. 453: the group sacrificed at 12 months should have contained 20 animals/sex/group instead of the 10 used in this study. Hematology at three and six months should have been performed and 20 animals/sex/group should have been examined instead of 10. Urinalysis and clinical biochemistry were not conducted.	Anonymous 57, 1995
8) TELONE* II SOIL FUMIGANT: 2-YEAR **INHALATION** CHRONIC TOXICITYONCOGENICITY	NOAEC: Chronic: 5 ppm (22.7 mg/kg/day) LOAEC:	This study is acceptable with some reservations.	Anonymous 55, 1985

STUDY IN MICE -- INTERIM REPORT: 6- AND 12- MONTH EXPOSURES 0, 5, 20 and 60 ppm (0, 22.7, 90.8, 272.4 mg/kg/d) Charles River CD-1 mice OECD TG 453 GLP Purity 92.1% 49.5% cis 42.6% trans	Chronic: 20 ppm (90.8 mg/kg/day) Hyperplasia and hypertrophy of the respiratory epithelium of the nasal turbinates	Deviations with respect OECD TG 453: there were only 10 (as opposed to 20) mice/sex/dose in the satellite groups for 6 and 12-month sacrifices; food consumption not conducted; ophthalmology not conducted; haematological examination was not conducted at 3 months; urinalysis was not conducted. Note: This study was conducted as a combined study; however, the reporting of satellite group data was done separately from the main/onco group.	
9) Telone Soil Fumigant **2-year inhalation** chronic toxicity/oncogenicity study in mice 0, 5, 20 and 60 ppm (0, 8.42, 33.70, 101.09mg/kg/d) B6C3F1 mice OECD TG 453 GLP Purity 92.1% 49.5% cis 42.6% trans	NOAEL: Chronic: 5 ppm (8.42 mg/kg/day) Oncogenic: 20 ppm (33.70 mg/kg/day) LOAEL: Chronic: 20 ppm (33.70mg/kg/day) Oncogenic: 60 ppm (101.09mg/kg/day) **Chronic LOAEL:** Lesions of the urinary bladder and nasal mucosa. Hyperplasia of the epithelial of the non-glandular portion of the stomach. Decreased body weight and modified organ weight **Carcinogenic LOAEL: Increase incidence of benign lung tumours.**	This study is acceptable with some reservations. Deviations with respect OECD 453: Only haematology and clinical chemistry of the 24-month exposure group were present in this report. Urinalyses was not conducted. Food consumption was not recorded. In period of holidays the treatment was not given. In some haematological parameters statistical comparison of means was not conducted.	Anonymous 56, 1987
10) National Toxicology Program (NTP). (1985) Toxicology and carcinogenesis studies of Telone II® (technical-grade 1,3-dichloropropene containing 1% epichlorohydrin as a stabilizer) in F344/N rats and B6C3F1 mice (gavage studies). U.S. Department of Health and Human Services Technical Report Series No. 269 B6C3F1 mice - 0, 50 and 100 mg/kg OECD Guideline – TG 451 GLP - yes	On the basis of urinary bladder and lung neoplasms in mice, the LOAEL for cancer in the study is 21.4 mg/kg (50 mg/kg/day × 3 days/7 days) and there is no NOAEL for mice	Not acceptable Deviations with respect to OECD TG 451 – dosing three times in a week, greatly reduced survival in the vehicle control group The current batches do not contain epichlorhydrin as stabilizer, thus the study results do not provided useful information for the current evaluation of test substance.	In: TOXICOLOGICAL REVIEW OF 1,3-DICHLOROPROPENE (CAS No. 542-75-6) - In Support of Summary Information on the Integrated Risk Information System (IRIS), *May 2000* - EPA/635/R-00/001 Draft Assessment Report, Spain, 2018

Table 62: Summary table of human data on carcinogenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
None available				

Table 63: Summary table of other studies relevant for carcinogenicity

Reference	Mechanism	Endpoint
Anonymous 68, 1977 1,3-Dichloropropene Purity 99.4% 51.3% cis 43.7% trans	The reaction of DD components with glutathione DD refers to 1,3-Dichloropropene	GSH level
Anonymous 62, 1985 92.1% / cis – 49.3% and trans – 42.8%	Inhalation, determining site of absorption in rats	Toxicokinetics following inhalation exposure in rats
Anonymous 61, 2015 Purity 97.5% 48.1% cis 49.4% trans	Inhalation, Absorption and Systemic Bioavailability in B6C3F1 Mice	Toxicokinetics following single inhalation exposure in mice
Anonymous 29, 2018 Purity 98.4% No information on isomer composition is available in the study report.	1,3-Dichloropropene: Steady State Pharmacokinetics Following Repeated Nose-Only Inhalation Exposure in Male B6C3F1 Mice with Plethysmography	Toxicokinetics following repeated inhalation exposure in mice
Anonymous 68, 1977 cis-1, 3-dicloropropene (Batch No. 20700) 99.4% purity. A mixture (51.3% cis-isomer and 43.7% trans-isomer) was also used.	The reaction of 1,3-D with glutathione (GSH)	Substrate (cis-, and trans-1,3-D) depletion.
Anonymous 60, 1982 Cis Sample Purity – 96% (cis - 94.1%; trans - 2.5%) Trans Sample Purity – 98% (trans – 97.39%; cis – 0.8%)	1,3-D: Effects on tissue non-protein sulfhydryl content and blood concentration time profile- probe study.	GSH Levels in liver, kidney, bladder, forestomach, glandular stomach.
Anonymous 59, 1985 Cis Sample Purity – 96% (cis - 94.1%; trans - 2.5%) Trans Sample Purity – 98% (trans – 97.39%; cis – 0.7%)	Pharmacokinetics, effect on tissue non-protein sulfhydryls, and macromolecular binding in rats and mice following single oral administration of 1,3- D.	Pharmacokinetics, Non-Protein Sulfhydryls (NPS) Depletion GSH level
Anonymous 66, 1996 Neither purity nor cis/trans ration was noted	Determination of glutathione transferase activities in several mammalian cell lines	GSH Level
Anonymous 65, 1997 96.5% purity. Cis – 49.87% and trans – 46.89%	Mechanism of tumorigenicity studies in male Rats.	DNA adduct formation *in vivo*
Anonymous 64, 1998 96.5% purity. Cis – 49.87% and trans – 46.89%	Supplemental Report for 1,3- D: Mechanism of tumorigenicity studies in male Rats and male mice	Histopathology examination
Anonymous 30, 2003 99.2% purity. Cis – 52.5% and trans – 46.7%	Examination of the mechanism of 1,3-D induced mouse lung and rat liver neoplasia	Glutathione S-transferase level

Anonymous 63, 2014 97.5% purity. Cis – 48.1% and trans – 49.4%	Evaluation of 1,3-D For Nuclear Receptor Activation Potential In Rat Primary Hepatocytes	Biomarker gene expression responses

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

Carcinogenicity: oral

Rats:

In the chronic toxicity and oncogenicity study with DD-92 (mixture consisting of 1,3-Dichloropropene E and Z isomers, and 1,2-Dichloropropane - 94.8% purity) in the rat via oral gavage administration by **Anonymous 53 (1998)**, 1,3-D was administered to Sprague Dawley rats (75/sex/group) at dose levels of 2, 10, and 25 mg/kg/day in corn oil for a period of at least 24 months. Control animals (75/sex) received the vehicle (corn oil) at the same dose volume as administered to the treated animals. After 12 months of treatment, up to ten animals/sex/group were sacrificed, and at termination of the study; 24 months for females and 23 months for males (due to the low survival rate seen in the low dose males during the study), all surviving animals were sacrificed. A high mortality rate occurred in all groups including controls as shown in the table below. Most deaths were accidental (due to intubation problems) and not related to treatment.

Table 64: Summary of survival data on the chronic toxicity and oncogenicity study with DD-92 (Anonymous 53, 1998)

Group	Percent survival [a]		Number of Animals					
	Males	Females	12-month sacrifice		Intercurrent deaths [b]		Terminal sacrifice	
			male	female	male	female	male	female
Vehicle control	34% (21/62)	24% (15/62)	9	7	45	53	21	15
2 mg/kg/day	17*% (10/58)	28% (16/57)	10	10	55	49	10	16
10 mg/kg/day	32% (19/60)	31% (19/61)	9	10	47	46	19	19
25 mg/kg/day	22% (14/63)	38% (24/63)	9	9	52	42	14	24

[a] These values exclude accidental deaths, [b] including Accidental deaths, *Statistically significantly different from control survivorship, $p \leq 0.05\%$

The mortality is very high for all groups, survival of all groups should be no less than 50% at 24 months for rats. Mean body weights and cumulative body weight gains of males dosed with 25 mg/kg bw/day were statistically significantly lower than concurrent control values, beginning at weeks 12 and 9 respectively, through week 72 of study. Mean body weights and body weights gains of all other groups of male and all treated females were comparable to control values. Haematology, clinical chemistry and urinalysis examined at months 6, 12, 18 and termination of study did not reveal any indications of adverse, treatment-related effects. All parameters evaluated were similar in all groups. Terminal organ and body weights, organ/body weight and organ/brain weight ratios, and absolute and relative organ weights of treated groups were considered comparable to control weights at month 12 and at termination of the study.

At 12- and 23/24-months, macroscopic findings occurred with comparable severity in rats from treatment and control groups. Some findings are considered to be incidental to treatment because they have been seen in control rats of similar strain and age used in other studies. However, the historical control data are not provided.

Microscopic morphologic abnormalities were observed in the forestomach of males and females at 10 and 25 mg/kg bw/day dosages at the 12-month interim sacrifice. A dose-related increased incidence of squamous cell hyperplasia and hyperkeratosis was observed for male and female rats, compared to controls. An increase in the severity of hyperkeratosis (from minimal to slight) was observed in males and females. The hyperplasia consisted of diffuse thickening of the epithelium; mitotic figures were not prominent and there was no evidence of atypia.

Table 65: Histopathologic Observations at 12-Months (Stomach) (Anonymous 53, 1998)

Sex	**Males**				**Females**			
Dose (mg/kg bw/day)	**0**	**2**	**10**	**25**	**0**	**2**	**10**	**25**
Number of rats examined	9	10	9	9	7	10	10	9

Fore stomach – squamous cell hyperplasia								
Minimal	2	-	3	7	-	1	2	3
Slight	1	-	2	-	1	-	2	5
Total	**3**	**0**	**5**	**7**	**1**	**1**	**4**	**8**
Fore stomach - hyperkeratosis								
Minimal	3	-	4	6	1	1	3	3
Slight	-	-	1	2	-	-	1	5
Total	**3**	**0**	**5**	**8**	**1**	**1**	**4**	**8**
Fore stomach – mixed inflammatory cell infiltrate								
Minimal	9	9	9	6	5	10	9	8
Slight	-	-	-	-	2	-	-	-
Total	**9**	**9**	**9**	**6**	**7**	**10**	**9**	**8**
Glandular mucosa – glands dilated								
Minimal	4	3	4	1	2	4	3	3
Slight	-	6	4	7	2	5	5	6
Moderate	3	-	-	-	3	1	1	0
Total	**7**	**9**	**8**	**8**	**7**	**10**	**9**	**9**

At the terminal sacrifice (23 months for males and 24 months for females) there were no neoplastic or non-neoplastic findings attributable to test substance administration. Other histopathologic findings in rats including scattered findings at various treatment levels which were slightly statistically significant when compared to controls, and findings which were the causes of death, were considered either due to ill health, or incidental and spontaneous in nature in rats of this strain and age, and were not directly associated with the test-article.

Incidence of neoplastic change

Sex	**Males**				**Females**			
Dosage (mg/kg bw/day	**0**	**2**	**10**	**25**	**0**	**2**	**10**	**25**
Number of Rats Examined	75	75	75	75	75	75	75	75
Adrenal	66	58	46	66	68	53	50	66
Adenoma, cortical	0	1	0	3	3	3	7	6
Carcinoma cortical					0	0	0	1
Pheochromocytoma, benign	11	5	6	14	5	3	1	3
Hemolymphoreticular neoplasm present	0	1	0	1	0	0	0	1
Artery, Aorta	66	55	47	66				
Hemolymphoreticular neoplasm present	0	0	0	1				
Bone	0	2	1	1				
Osteosarcoma	0	1	0	0				
Hemolymphoreticular neoplasm present	0	1	0	0				
Bone Marrow, Sternum	65	55	47	65				
Hemolymphoreticular neoplasm present	1	2	1	3				
Bone, Femur								
Hemolymphoreticular neoplasm present	0	1	0	0				
Brain	66	55	47	66	68	49	45	66
Astrocytoma	1	1	1	1				
Granular cell tumor					1	0	0	0
Leukemic cells, intravascular	0	1	0	0				
Meningioma	1	0	0	0	1	0	0	1
Metastic tumor					1	2	0	3
Oligodendroma	0	0	1	0				
Ear					18	7	12	9
Fibrous histiocytoma, benign					0	2	0	0
Melanoma, benign					0	0	1	0
Eye								
Hemolymphoreticular neoplasm present	0	1	1	0				
Gland, Mammary	1	7	5	3	67	56	52	66
Adenocarcinoma	0	0	0	1	7	10	10	4
Adenoma					4	2	0	5

Sex	Males				Females			
Dosage (mg/kg bw/day	**0**	**2**	**10**	**25**	**0**	**2**	**10**	**25**
Carcinoma					0	2	0	1
Carcinosarcoma					0	1	1	1
Fibroadenoma	1	1	1	0	22	16	18	31
Hemolymphoreticular neoplasm present	0	0	1	1	0	0	1	0
Gland, Salivary	66	55	47	66	68	48	44	66
Adencocarcinoma					0	0	1	0
Hemolymphoreticular neoplasm present	0	0	0	1	0	0	1	0
Gland, Zymbal's	1	1	1	2				
Adenoma	0	0	0	1				
Carcinoma	0	0	1	1				
Squamous cell carcinoma	1	1	0	0				
Heart	66	56	47	66	68	48	45	66
Leukemic cells, intravascular	0	1	0	0				
Metastic tumor					1	1	0	0
Hemolymphoreticular neoplasm present	0	0	0	1	1	0	0	1
Intestine, Large, Cecum	64	65	66	66	68	60	63	66
Carcinoma					0	1	0	0
Hemolymphoreticular neoplasm present	1	0	0	0	1	0	0	0
Intestine, Large, Colon					68	59	64	66
Leiomyosarcoma					0	0	1	0
Intestine Small, Duodenum					68	59	64	66
Hemolymphoreticular neoplasm present					0	0	0	1
Intestine, Small, Ileum	65	65	66	66	68	59	64	66
Hemangiosarcoma	1	0	0	0				
Hemolymphoreticular neoplasm present					1	0	0	0
Kidney	66	65	66	66	68	65	64	66
Adenoma	0	0	0	1				
Lipoma					0	0	1	0
Hemolymphoreticular neoplasm present	1	1	1	1	2	0	0	1
Larynx	0	0	0	1				
Hemolymphoreticular neoplasm present	0	0	0	1				
Liver	66	65	66	66	68	65	65	66
Adenoma	2	1	2	1	0	0	0	1
Carcinoma	2	1	1	0				
Cholangiocarcinoma	0	0	1	0				
Hemangiosarcoma					0	0	1	0
Hemolymphoreticular neoplasm present	2	4	3	4	2	0	2	1
Lung	66	65	66	66	68	65	65	66
Adenoma					1	0	0	0
Metastic tumor	1	0	0	0	3	3	1	1
Hemolymphoreticular neoplasm present	2	3	3	5	2	0	3	0
Lymph Node, Abdominal	0	4	1	0				
Hemolymphoreticular neoplasm present	0	2	0	0				
Lymph Node, Axillary					0	2	0	0
Metastic tumor					0	1	0	0
Lymph Node, Inquinal	0	0	1	0				
Hemolymphoreticular neoplasm present	0	0	1	0				
Lymph Node, Mediastinal	2	3	2	1				
Hemolymphoreticular neoplasm present	1	0	1	0				
Lymph Node, Mesenteric	65	55	47	66	67	48	45	66
Fibrosarcoma	1	0	0	0				
Hemangioma	0	0	0	1	0	1	0	0
Hemangiosarcoma	1	0	0	0				
Lymphangioma	1	0	0	0				
Metastatic tumor	0	1	0	0				
Hemolymphoreticular neoplasm present	2	1	2	1	1	0	1	2

Sex	Males				Females			
Dosage (mg/kg bw/day	**0**	**2**	**10**	**25**	**0**	**2**	**10**	**25**
Lymph Node, Submaxillary	64	55	47	64	68	49	47	66
Hemolymphoreticular neoplasm present	1	2	1	1	1	0	0	1
Hemolymphoreticular neoplasm present	1	1	0	1				
Muscle, Diaphragm					2	0	0	0
Rhabdomyosarcoma					1	0	0	0
Hemolymphoreticular neoplasm present					1	0	0	0
Muscle, Skeletal					67	48	43	65
Hemolymphoreticular neoplasm present					1	0	1	0
Nerve, Sciatic	66	55	47	56				
Leukemic cells, intravascular	0	1	0	0				
Hemolymphoreticular neoplasm present	0	1	1	0				
Ovary					68	54	47	66
Adenocarcinoma					1	0	0	0
Granulosa cell tumor, benign					1	1	0	2
Hemolymphoreticular neoplasm present					1	0	0	0
Oviduct					67	48	44	64
Hemolymphoreticular neoplasm present					1	0	0	0
Pancreas	66	58	48	65	68	49	46	66
Adenoma, exocrine	3	2	3	4				
Carcinoma, exocrine	0	1	0	0				
Hemangioosarcoma					0	0	1	0
Islet cell adenoma	2	3	0	3	6	4	1	1
Hemolymphoreticular neoplasm present	0	0	0	1	1	0	1	0
Parathyroid	65	53	46	62	65	49	44	65
Adenoma	0	0	0	2	1	2	0	1
Pituitary	66	56	56	66	68	62	61	66
Adenoma	27	25	28	25	5	51	46	48
Carcinoma					1	2	0	3
Craniopharyngioma	1	0	0	0				
Prostate	66	54	47	66				
Hemolymphoreticular neoplasm present	0	0	1	0				
Skin	66	59	54	66	68	51	47	66
Adenoma, sebaceous cell					0	0	1	0
Fibroma	0	0	1	1	0	0	0	3
Fibrosarcoma					1	0	0	0
Fibrous histiocytoma, benign	0	3	1	1	0	0	0	1
Fibrous histiocytoma, malignant	0	1	0	0	1	0	0	0
Hemangiosarcoma	0	1	0	0				
Keratoacanthoma	3	2	1	2				
Lipoma	0	2	2	1	2	0	1	1
Myxosarcoma	0	0	1	0				
Papilloma	1	0	1	0	0	0	0	1
Hemolymphoreticular neoplasm present	1	2	1	3	0	0	2	0
Skin, Hind-foot	12	16	19	17				
Papilloma	0	0	1	0				
Skin, Tail	15	23	13	12				
Fibrous histiocytoma, benign	1	0	0	0				
Spleen	66	55	47	66	68	48	48	66
Hemangiosarcoma	1	1	0	1				
Hemolymphoreticular neoplasm present	1	2	2	2	0	0	0	1
Stomach	65	65	66	65	68	65	64	66
Metastic tumor					1	0	0	0
Hemolymphoreticular neoplasm present	0	1	1	0	1	0	0	1
Testis	66	57	48	66				
Leydig cell (interstitial cell) adenoma	0	1	0	1				
Hemolymphoreticular neoplasm present	0	1	0	0				

Sex	Males				Females			
Dosage (mg/kg bw/day	**0**	**2**	**10**	**25**	**0**	**2**	**10**	**25**
Thymus	62	49	40	56	60	42	41	58
Mast cell tumor	0	0	0	1				
Metastatic tumor					1	1	0	0
Hemolymphoreticular neoplasm present	3	1	1	1	1	0	1	1
Thyroid	66	54	47	66	68	49	45	65
Adenoma. C cell	0	1	0	0	1	4	3	2
Adenoma. Follicular cell	1	1	0	1				
Hemolymphoreticular neoplasm present	0	0	1	0	0	0	1	0
Tissue, Adipose	2	3	0	2	4	2	3	5
Lipoma					1	0	1	1
Liposarcoma	0	1	0	0				
Urinary Bladder	66	54	47	66				
Carcinoma, tranitional cell	0	0	0	1				
Hemolymphoreticular neoplasm present	0	1	0	0				
Leiomyoma					1	0	0	0
Papilla					0	0	0	1
Uterus					68	54	49	66
Adenocarcinoma					0	0	0	1
Granular cell tumor					1	0	0	0
Leiomyosarcoma					0	0	0	1
Polp					2	3	1	0
Scarcoma, stromal					0	1	0	0
Hemolymphoreticular neoplasm present					2	0	0	0
Vagina					68	49	44	66
Carcinosarcoma					0	0	0	1
Granular cell tumor					0	1	0	0
Hemangioma					0	1	0	0
Hemolymphoreticular System [# affected]	6	4	3	7	2	0	3	3
Lymphosarcoma	2	2	2	2	0	0	0	2
Leukemia, granulocytic					0	0	0	1
Sarcoma, histiocytic	4	2	1	5	2	0	3	0

Specific statistically significant findings included foreign material in the lungs of males in all treatment groups. This was presumed to be aspirated test material upon withdrawal of the gavage tube; there was no dose relationship (i.e. 20/75, 33/75, 31/75, and 32/75 at 0, 2, 10, and 25 mg/kg dosages respectively); there were no associated adverse histopathological findings in the lungs; and the incidence in the high-dose females (22/75) is lower than in the control females (27/75).

A slight, but statistically increased incidence of chronic progressive nephropathy was observed in the kidneys of high-dose males (69/75 versus controls 59/75). This increased incidence was not considered to be treatment-related because both of these incidences were compatible with that reported in rats of this strain and age (data not reported). Moreover, chronic progressive nephropathy occurred in almost all male rats at the interim sacrifice (9/9 controls and 8/9 high-dose males) as well as the terminal sacrifice (20/21 controls and 14/14 high-dose males).

Lastly, a slight but statistical increased incidence of thymic involution was observed in high dose females (63/66) versus controls (54/64). This increase was not considered to be treatment-related because this observation is a quite common incidental finding in old and/or debilitated animals such as those which died on study (40/45 controls and 36/37 high-dose females). Moreover, thymic involution occurred in almost all female rats at the terminal sacrifice (11/15 control and 20/21 high dose females).

It was concluded that the no-observed-effect-level (NOAEL) for chronic toxicity was 2 mg/kg/day, based on the increased incidence and/or severity of hyperplasia and hyperkeratosis of the stratified squamous epithelium lining the forestomach observed in males and females treated with 10 mg/kg/day. 1,3-D did not result in any

oncogenic effects. Based on this study, the NOEL for potential oncogenicity of 1,3-D was 25 mg/kg bw/day, the highest dose tested. However, the high mortality rate on this study is likely to have compromised its reliability.

In 1995, **Anonymous 54**, tested 1,3-D microencapsulated in a starch-sucrose (80:20) matrix (Telone II) purity of 96.0% (50.7% cis/45.1% trans), in a combined chronic toxicity and oncogenicity test in Fischer 344 rats. Sixty Fischer 344 rats/sex/dose level were administered test diets formulated to supply dosages of 0, 2.5, 12.5 and 25 mg/kg bw/day Telone II Soil Fumigant. Fifty rats/sex/dose level were scheduled to receive the test material for two years. Ten rats/sex/dose level were designated as a satellite group that was scheduled for sacrifice at 12 months. Sixty Fischer 344 rats/sex/dose level were administered test diets formulated to supply dosages of 0, 2.5, 12.5 and 25 mg/kg bw/day Telone II Soil Fumigant. Fifty rats/sex/dose level were scheduled to receive the test material for two years. Ten rats/sex/dose level were designated as a satellite group that was scheduled for sacrifice at 12 months. Mortality rate was acceptable on this study.

Table 66: Summary of survival data on the chronic toxicity and oncogenicity study with Telone II (Anonymous 54, 1995)

Percent survival		
Group	Males	Females
Vehicle control	26% (13/50)	22% (11/50)
2.5 mg/kg/day	32% (16/50)	30% (15/50)
12.5 mg/kg/day	32% (16/50)	38% (19/50)
25 mg/kg/day	32% (16/50)	22% (11/50)

The body weights of males and females at 25 mg/kg/day were statistically significantly lower than controls over the entire dosing period. At 12.5 mg/kg/day, statistically significantly lower body weights were observed in males from week-13 of dosing and onwards, and in females from week-60 onwards, and feed consumption values for high dose group male and female rats were decreased relative to controls consistent with their lower body weights. Body weights were decreased as much as 15-16% and body weight gains were suppressed an average of 17-19% in rats ingesting 25 mg/kg/day Telone 11. At 12.5 mg/kg/day these parameters were also decreased up to 9% and 14%, respectively, in males over a majority of the dosing period and up to 9% and 12%, respectively, in females over the last 3-4 months of dosing.

Body Weights

Dose (mg/kg/day)	**0**		**2.5**		**12.5**		**25**	
Days on Test	**Male**	**Fem.**	**Male**	**Fem.**	**Male**	**Fem.**	**Male**	**Fem.**
-3	157.7	119.7	158.0 (0,19%)	120.5 (0,67%)	157.9 (0,13%)	120.0 (0,25%)	157.2 (-0,32%)	119.7 (0,00%)
8	200.9	136.6	203.0 (1,05%)	136.8 (0,15%)	201.6 (0,35%)	135.9 (-0,51%)	195.7* (-2,59%)	133.7* (-2,12%)
15	215.1	141.5	218.8	141.5	214.5	139.8	206.4*	137.0*
22	233.1	151.3	235.7	152.1	231.3	150.9	222.0*	146.7*
29	243.6	155.5	247.7	156.6	241.5	155.9	232.9*	150.8*
36	252.5	161.6	257.5	162.8	251.4	162.7	242.4*	157.3*
43	257.9	162.0	262.7	162.5	255.6	161.4	245.6*	156.4*
50	268.8	168.9	272.3	169.2	265.5	167.8	253.5*	161.9*
57	278.8	172.4	280.8 (0,72%)	171.7 (-0,41%)	274.5 (-1,54%)	170.1 (-1,33%)	261.8* (-6,10%)	164.3* (-4,70%)
64	282.8	176.0	284.6	175.4	277.9	174.7	266.3*	167.4*
71	291.7	176.0	291.9	177.3	286.0	176.5	273.2*	168.5*
78	294.9	179.1	294.9	179.2	288.7	176.9	274.0*	168.3*
85	302.8	179.4	301.2	179.0	295.4	178.9	277.6*	170.0*
92	307.2	184.6	304.2 (-0,98%)	184.0 (-0,33%)	297.4* (-3,19%)	181.3 (-1,79%)	279.7* (-8,95%)	173.8* (-5,85%)
120	321.7	187.4	314.6	187.2	305.8*	185.0	288.5*	176.5*

Dose (mg/kg/day)	0		2.5		12.5		25	
Days on Test	**Male**	**Fem.**	**Male**	**Fem.**	**Male**	**Fem.**	**Male**	**Fem.**
145	337.0	194.0	330.8	192.3	319.7*	190.3	301.2*	181.7*
173	356.6	202.5	350.2 (-1,79%)	198.3 (-2,07%)	335.8* (-5,83%)	195.8 (-3,31%)	318.0* (-10,82%)	187.5$ (-7,41%)
201	368.9	202.1	363.9	200.4	351.3*	200.2	326.8*	190.7*
229	379.4	204.8	373.3	205.4	355.5$	204.6	333.1$	193.5*
257	389.2	210.0	385.4	208.0	267.7*	208.3	344.1*	197.9*
285	400.8	211.6	396.4	211.1	378.9*	210.8	347.1*	199.2*
313	405.4	215.3	399.8	212.6	380.5*	210.1	351.9*	200.1*
341	411.2	217.4	406.3	217.0	385.6*	216.6	355.2*	203.7$
369	415.5	218.9	411.9 (-0,87%)	219.6 (0,32%)	389.9* (-6,16%)	215.8 (-1,42%)	355.1* (-14,54%)	203.6$ (-6,99%)
397	420.7	222.9	412.3	221.5	390.3*	220.4	360.2*	207.3*
425	422.4	226.7	419.0	224.3	394.2*	218.7*	354.6*	208.4*
453	419.4	233.4	413.5 (-1,41%)	231.8 (-0,69%)	390.4* (-6,91%)	229.6 (-1,63%)	359.6* (-14,26%)	215.0* (-7,88%)
481	416.7	241.1	412.7	237.6	387.5*	236.5	358.9*	218.6*
509	420.1	250.8	413.9	247.8	389.5*	245.3	359.8*	224.0*
537	422.6	258.6	417.6	256.4	389.6*	253.2	362.6*	232.0*
565	414.1	262.6	407.4 (-1,62%)	261.0 (-0,61%)	378.3* (-8,65%)	255.8 (-2,59%)	354.1* (-14,49%)	230.3* (-12,30%)
593	411.7	266.7	407.4	266.5	385.0*	258.0	354.7*	234.4*
621	409.3	273.9	405.2	268.8	379.4*	262.6	353.1*	238.9*
549	404.6	283.3	401.0	277.8	375.2*	264.3*	352.3*	244.0*
677	394.0	283.4	386.4	281.5	371.1$	264.1*	349.1$	242.1*
705	385.4	281.5	379.5	282.2	359.7*	263.7*	339.6*	244.8*
727	384.2	284.5	374.3 (-2,58%)	280.5 (-1,41%)	364.0 (-5,26%)	260.4* (-8,47%)	335.1* (-12,78%)	244.3* (-14,13%)

*Statistically different from control mean by Dunnett's test, alpha = 0.05.

$Statistically different from control mean by Wilcoxon's test, alpha = 0.05.

Organ Weights

	0 mg/kg/day		2.5 mg/kg/day		12.5 mg/kg/day		25 mg/kg/day	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
12 Months								
Final B.W	386.3	208.3	389.5	206.6	367.6	199.1	337.9*	186.9$
Brain(g/100)	0.527	0.885	0.529 (0.38%)	0.891 (0,68%)	0.563* (6.83%)	0.922 (4.18%)	0.601* (14.04%)	0.976* (10.28%)
Heart(g)	1.002	0.650	0.994 (-0.80%)	0.643 (-1.28)	0.978 (-2.40%)	0.646 (-0.62%)	0.919* (-8.28%)	0.629 (-3.23%)
Heart(g/100)	0.260	0.314	0.256 (-1,54%)	0.312 (-0,64%)	0.266 (2,31%)	0.325 (3,50%)	0.272 (4,62%)	0.337* (7,32%)
Kidney(g/100)	0.601	0.675	0.593 (-1,33%)	0.697 (3,26%)	0.603 (0,33%)	0.705 (4,00%)	0.639* (6,32%)	0.726* (7,56%)
Liver(g)	9.370	5.370	9.608 (2,54%)	5.42 (0,93%)	9.098 (-2,90%)	5.222 (-2,76%)	8.465* (-9,66%)	5.118 (-4,69%)
Liver(g/100)	2.426	2.582	2.464 (1,57%)	2.621 (1,51%)	2.474 (1,98%)	2.623 (1,59%)	2.507 (3,34%)	2.738* (6,04%)
24 Months								
Final B.W.(g)	357.0	265.1	347.6 (-2.63%)	261.4 (-1,40%)	332.2* (-6.95%)	246.0* (-7,20%)	310.8* (-12.94%)	226.8* (-14,45%)
Adrenals(g)	0.065	0.065	0.066 (1,54%)	0.063 (-3,08%)	0.060$ (-7,69%)	0.064 (-1,54%)	0.057$ (-12,31%)	0.061$ (-6,15%)
Brain(abs)	2.063	1.863	2.084 (1,02%)	1.877 (0,75%)	2.047 (-0,78%)	1.878 (0,81%)	2.069 (0,29%)	1.911* (2,58%)
Brain(rel)	0.582	0.714	0.614	0.723	0.623$	0.772$	0.675$	0.852$

			(5,50%)	(1,26%)	(7,04%)	(8,12%)	(15,98%)	(19,33%)
Heart(abs)	1.061	0.805	1.045 (-1,51%)	0.784 (-2,61%)	1.017 (-4,15%)	0.789 (-1,99%)	0.968* (-8,77%)	0.782$ (-2,86%)
Heart(rel)	0.299	0.307	0.305 (2,01%)	0.302 (-1,63%)	0.309 (3,34%)	0.324$ (5,54%)	0.313 (4,68%)	0.348$ (13,36%)
Kidneys(g/100)	0.786	0.717	0.815 (3,69%)	0.744 (3,77%)	0.819$ (4,20%)	0.791* (10,32%)	0.882$ (12,21%)	0.809* (12,83%)
Liver(g)	11.155	7.074	11.446 (2,61%)	7.273 (2,81%)	10.813 (-3,07%)	7.494 (5,94%)	9.716$ (-12,90%)	6.466$ (-8,59%)
Liver(g/100)	3.145	2.683	3.318 (5,50%)	2.778 (3,54%)	3.253 (3,43%)	3.091$ (15,21%)	3.132 (-0,41%)	2.880$ (7,34%)

*Statistically different from control mean by Dunnett's test, alpha = 0.05.
$Statistically different from control mean by Wilcoxon's test, alpha = 0.05.

Microscopic examination revealed a minimal degree of basal cell hyperplasia of the non-glandular stomach mucosa in intermediate and high dose group males and females which displayed no evidence of progression from the 12-month time point to the 24-month time point. An increased incidence of foci of altered cells in the liver, a common age-related observation in Fischer 344 rats, was also noted in all treated groups of rats following 24 months dosing. The latter observation, however, was considered of equivocal toxicologic significance.

Table 67: Summary of microscopic examination non-neoplastic findings for relevant tissues (Anonymous 54, 1995)

Sex	**Males**				**Females**			
Dose (mg/kg bw/day)	**0**	**2.5**	**12.5**	**25**	**0**	**2.5**	**12.5**	**25**
12 MONTHS								
Number of rats examined	10	10	10	10	10	10	10	10
Stomach – No. of tissues examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Non-glandular mucosa, basal cell hyperplasia								
Very slight	0	1	5	6	0	0	2	7
Slight	0	0	2	4	0	0	1	2
Total	**0**	**1**	**7**	**10**	**0**	**0**	**3**	**9**
24 MONTHS								
Number of rats examined	50	50	50	50	50	50	50	50
Stomach – No. of tissues examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Non-glandular mucosa, basal cell hyperplasia								
Very slight	3	3	19*^{T}	18*^{T}	0	1	19*^{T}	33*^{T}
Slight	0	0	1	12*	0	0	1	4
Total	**3**	**3**	**20***	**30*^{T}**	**0**	**1**	**20*^{T}**	**37*^{T}**
Liver – No. of Tissues Examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Focus(I) of altered cells - eosinophilic, hepatocellular								
Very slight	29	25	18*	11*^{T}	12	24*	20	32*^{T}
Slight	3	11*	23*	24*^{T}	0	3	3	1
Moderate	0	0	2	1	0	0	0	0
Total	**32**	**36**	**43**	**36**	**12**	**27**	**23**	**33*^{T}**
Hyperplasia-regenerative, hepatocellular, focal	0	2	0	3	0	1	0	1
Hyperplasia-regenerative, hepatocellular, multifocal	5	2	0	4	0	1	5	1
Total	**5**	**4**	**0**	**7**	**0**	**2**	**5**	**2**

* Statistically different from control mean by Yates Chi-squared pairwise test, Alpha = 0.10, two-sided, Alpha = 0.05, one-sided.
^{T} Linear trend test by Cochrane-Armitage linear trend test, Alpha = 0.02, two-sided, Alpha = 0.01, one-sided.

The incidence of benign liver tumours (hepatocellular adenomas) was statistically significantly increased in males and females by a trend test. Pairwise comparison of tumour incidence, however, indicated a statistically significant increase only in high dose male rats. No treatment-related increase in the incidence of other tumour types was observed.

Table 68: Summary of microscopic examination neoplastic findings for relevant tissues (Anonymous 54, 1995)

Sex	**Males**				**Females**			
Dose (mg/kg bw/day)	**0**	**2.5**	**12.5**	**25**	**0**	**2.5**	**12.5**	**25**
24 MONTHS – neoplastic changes in liver								

Sex	Males				Females			
Dose (mg/kg bw/day)	**0**	**2.5**	**12.5**	**25**	**0**	**2.5**	**12.5**	**25**
Liver – No. of Tissues Examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Adenoma, bile duct(s), benign, primary:	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
Adenoma, hepatocellular, benign, primary:								
one	1	1	3	8*[T]	0	0	0	4
two	1	0	2	1	0	0	0	0
three	0	0	1	0	0	0	0	0
Any number	**2**	**1**	**6**	**9*[T]**	**0**	**0**	**0**	**4 [T]**
Carcinoma, hepatocellular, malignant, primary, no metastasis	0	0	0	1	0	0	0	0
Haemangioma, benign, primary:	0	0	0	0	0	0	0	1
Hemangiosarcoma, malignant, primary, no metastasis:	0	0	0	1	0	0	0	0
Rhabdomyosarcoma, (skeletal muscle), malignant, secondary:	1	0	0	0	0	0	0	0
Undifferentiated sarcoma, malignant, primary, no metastasis:	0	0	0	1	0	0	0	0
Histiocytic sarcoma, malignant, primary, no metastasis	0	0	0	1	0	0	0	0

* Statistically different from control mean by Yates Chi-squared pairwise test, Alpha = 0.10, two-sided, Alpha = 0.05, one-sided.
[T] Linear trend test by Cochrane-Armitage linear trend test, Alpha = 0.02, two-sided, Alpha = 0.01, one-sided.

In the paper by **Anonymous 67**, 2000 (summary of results obtained in the study of Anonymous 55 (1985)), the authors included the historical background incidence for hepatocellular adenoma in six studies with F344 rats from the same laboratory. For female rats, the incidence for hepatocellular adenomas varied from 0/50 to 3/50. For male rats the historical background incidence varied from 0/50 to 2/50. Therefore, the increased incidence of hepatocellular adenomas in males given 12.5 or 25 mg/kg/day and females given 25 mg/kg/ day are outside the historical control range.

The no-observed-adverse-effect level for systemic chronic toxicity of Telone II in the Fischer 344 rat of 2.5 mg/kg/day. The observations in the stomachs (basal cell hyperplasia of the non-glandular mucosa of the stomach in males and females ingesting 12.5 or 25 mg/kg bw/day Telone II) and in the liver (hepatocellular adenoma in males ingesting 25 mg/kg bw/day and 12.5 mg/kg bw/day Telone II) can be considered the definitive morphologic effects of treatment associated with these chronic dose levels in the F344 rat.

The relationship of foci of altered cells to neoplasia is unclear. Foci are sometimes considered by some to be preneoplastic lesions. However, other studies indicate that they are reversible lesions while still others suggest that they are non-neoplastic end stage lesions (Goodman *et al.*, 1994).

Based on the absence of these definitive-related effects in male and female rats ingesting the low dose level, and equivocal significance of the liver foci of altered cells described above, the low dose level of 2.5 mg/kg bw/day was interpreted as the systemic and oncogenic NOAEL for Telone II in the Fischer 344 rat.

NTP, 1985 (and references therein)

Toxicology and carcinogenesis ingestion studies of Telone II® (88%–90% 1,3-dichloropropene, 2.5% 1,2-dichloropropane, 1.5% trichloropropene isomer, and 1% epichlorohydrin) were conducted by administering the commercial-grade formulation in corn oil by gavage to groups of 52 male and 52 female F344/N rats at doses of 0, 25, or 50 mg/kg, three times weekly for 104 weeks. Ancillary studies were conducted in which additional dose groups containing five male and five female rats were killed after receiving Telone II® for 9, 16, 21, 24, or 27 months. At study termination, there were no toxicologically significant changes in body weight. Survival in treated rats was comparable to that in vehicle controls.

Nonneoplastic lesions. In rats, increases in hyperplastic lesions of the basal layer of the squamous epithelium in the forestomach were observed in both sexes at 25 and 50 mg/kg. These lesions were duration dependent and were seen as early as 9–16 months after treatment began. The incidence for males was significantly increased in the 2-year and ancillary studies combined at both doses, whereas the incidence for females was significant only at 50 mg/kg. Table 69 shows the incidence for these effects in the 2-year study.

Table 70: Incidence of forestomach and liver lesions in rats in the ancillary studies from NTP (1985) 2-year study

	Vehicle Control (a)					25 mg/kg (a)					50 mg/kg (a)				
	9	16	21	24	27	9	16	21	24	27	9	16	21	24	27
	(months on study)					(months on study)					(months on study)				
MALE															
Stomach: Basal Cell Hyperplasia	0/5	0/5	1/5	0/5	0/5	0/5	1/5	3/5	3/5	1/5	1/5	5/5	4/5	4/5	4/5
Stomach: Squamous Cell Papilloma	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	2/5
Liver: Neoplastic Nodule	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/4	0/5	0/5	0/5	0/5	0/5	1/5	0/5
FEMALE															
Stomach: Basal Cell Hyperplasia	0/5	0/5	0/5	0/5	0/3	0/5	2/5	2/5	1/5	0/5	0/5	5/5	5/5	4/5	5/5
Stomach: Squamous Cell Papilloma	0/5	0/5	0/5	0/5	0/3	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	5/5
Liver: Neoplastic Nodule	0/5	0/5	0/5	0/5	0/3	0/5	0/5	0/5	2/5	0/5	0/5	0/5	0/5	0/5	2/5

(a) Scheduled kills

The LOAEL for rats, based on hyperplastic lesions of the forestomach, is 25 mg/kg. Averaging the exposure over 7 days yields a LOAEL of 10.7 mg/kg/day.

Neoplastic lesions. In rats, an increase in the incidence of forestomach tumors, mainly benign tumors, was observed. In males there was a statistically significant increase in the 50 mg/kg group for squamous cell papilloma and squamous cell papillomas and carcinomas combined. In female rats, a statistically significant increase was only observed for squamous cell papillomas at 50 mg/kg when the 2-year and ancillary studies were combined. Although the nonneoplastic lesions of the forestomach developed within 1 year of exposure, the neoplastic lesions did not appear until 24 months after exposure began. The incidence of forestomach tumors at 25 mg/kg was similar to controls for both sexes.

Table 710: Incidence[a] of selected cancer and noncancer effects in rats from NTP (1985) 2-year study

	Males			Females		
Lesion	0 mg/kg/day	25 mg/kg/day	50 mg/kg/day	0 mg/kg/day	25 mg/kg/day	50 mg/kg/day
Basal cell or epithelial hyperplasia of stomach	2/52	5/52	13/52	1/52	0/52	16/52
Squamous cell papilloma/carcinoma of forestomach	1/49	1/48	13/50	0/47	2/45	3/48
Liver neoplastic nodule	1/49	6/48	7/50	6/46	6/42	10/49
Hepatocellular carcinoma	0/49	0/48	1/50	0/46	0/42	0/49

[a] 52 rats started in each group. For tumor incidence, rats that died before the first tumor appeared were omitted from the number at risk.

Neoplastic nodules, classified as nodular hyperplasia and described as “small focal lesions causing only minimal compression, with little or no cytologic atypia in livers, or with toxic or anoxic hepatic changes,” (NTP, 1985) were noted in the livers of male rats. In the current classification scheme, neoplastic nodules are classified as adenomas (Maronpot *et al.*, 1986 [in NTP, 1985]). The increased incidence was statistically significant for the 25 and 50 mg/kg doses. One male rat in the 50 mg/kg group exhibited a hepatocellular carcinoma after 2 years of exposure. In light of the occurrence of a liver carcinoma, the biological significance of the neoplastic liver nodules is increased. There were no statistically significant increases in liver tumors in female rats. Anonymous 54 (1995) also observed liver adenomas in male rats receiving 25 mg/kg/day 1,3-dichloropropene in the diet.

From the rat study, NTP (1985) and references therein, concluded that there was clear evidence of carcinogenicity in male rats, based on the combined incidences of squamous cell papillomas and carcinomas of the forestomach and the increased incidence of liver adenoma. In female rats, there was some evidence of carcinogenicity, based on the increased incidence of squamous cell papillomas of the forestomach. However, NTP (1985) recognized that epichlorohydrin, a stabilizer present in Telone II®, may be partially responsible for the hyperplasia and squamous cell papilloma/carcinoma, at least in rat forestomach. NTP states this is plausible because the same types of lesions were found by Konishi et al. (1980) [in NTP, 1985] in a drinking water study with Wistar rats, and because the local exposure of the stomach to epichlorohydrin may have reached a concentration similar to that administered by Konishi et al. (1980). Subsequent to the NTP (1985) study, a 2-year gavage study with epichlorohydrin was published (Wester et al., 1985 [in NTP, 1985]). Wester et al. (1985) observed a 28% incidence of forestomach papilloma/carcinoma in male Wistar rats and a 15% incidence in females given 1.4 mg/kg/day epichlorohydrin. In comparison, NTP (1985) found a 22% incidence in males and a 10% incidence in females at 2.1 mg/kg/day (50 mg/kg/day Telone II® × 0.1 epichlorohydrin × 3 days/7 days). The chronic feeding study by Anonymous 54, (1995) testing Telone II doses up to 25 mg/kg bw/day, which did not include epichlorohydrin, reported forestomach hyperplasia in rats but no carcinomas or papillomas.

On the basis of forestomach and liver neoplasms in rats, the LOAEL for cancer in the NTP (1985) study is 21.4 mg/kg (50 mg/kg/day × 3 days/7 days) and the NOAEL for rats is 10.7 mg/kg (25 mg/kg/day × 3 days/7 days).

Mice:

In an oncogenicity study (Anonymous 52, 1997), DD-92 (a mixture consisting of 1,3-Dichloropropene (E) and (Z) isomers - 94.8% purity), was administered orally, via intubation (oral gavage), to CD-1 albino mice (65/sex/group) at dose levels of 2, 10, and 25 mg/kg/day (at a constant dose volume of 5 ml/kg/dose) in corn oil for a period of at least 18 months. Control animals (65/sex) received the vehicle (corn oil) at the same dose volume as administered to the treated animals. Food and water were available *ad libitum*. Animals were examined twice daily. The study was GLP compliant and conducted in accordance with OECD TG 451, except for the following deviation; Haematology should have been performed for all animals instead of 10 used in this study.

A detailed physical examination was performed twice pre-test and weekly thereafter. The body weights and food consumption were recorded pre-test, weekly through 16 weeks and monthly thereafter. Haematology parameters were examined for 10 animals/sex/group at month 12 and at termination. All survivors were sacrificed at termination (18 months) and selected organs were weighed, and organ/body weight and organ/brain weight ratios calculated. Gross evaluations were performed and selected tissues from all animals were preserved. Selected tissues were examined microscopically for animals sacrificed at termination in control and high dose groups, and for all animals that died during the study. In addition, kidneys, liver, lungs, large and small intestines, stomach, urinary bladder and gross lesions were examined for all animals in all groups.

There were no statistically significant differences in survivorship as compared to controls for males or females at any dose level.

Table 72: Summary of survival from the oncogenicity study in mice (Anonymous 52, 1997)

Percent survival [a]		
Group	Males	Females
Vehicle control	79% (50/63)	82% (50/61)
2 mg/kg/day	72% (43/60)	72% (42/58)
10 mg/kg/day	76% (48/63)	70% (43/61)
25 mg/kg/day	70% (45/64)	66% (41/62)

[a] Animals which died accidentally or were sacrificed for humane reasons were excluded from potential survivors and from total dead.

There were no physical observations related with treatment. Mean body weights of all treated groups were comparable to control weights during the study. Haematology parameters examined at month 12 and termination of study did not reveal any adverse effects due to administration of DD-92. Absolute and relative organ weights of treated groups were comparable to control weights at termination of the study, and there were no macroscopic morphologic findings related to treatment with DD-92.

Treatment-related histopathological changes were seen in the urinary bladder of females at 25 mg/kg/day; Statistically significantly increased non-neoplastic microscopic findings consisted of transitional cell hyperplasia, and hyaline change of the lamina propria, which were considered to reflect responses to chronic irritation. Other treatment related findings in the urinary bladder of high-dose females included stromal hyperplasia, stromal hypertrophy and accumulation of brown pigment in reticuloendothelial cells.

Table 73: Summary of non-neoplastic findings in mice (Anonymous 52, 1997)

Sex	**Males**				**Females**			
Dose (mg/kg bw/day)	**0**	**2**	**10**	**25**	**0**	**2**	**10**	**25**
Number of mice examined	**65**	**63**	**64**	**65**	**65**	**63**	**61**	**65**
Hyalin change								
Minimal	0	0	1	3	1	0	0	8
Slight	0	0	1	3	0	0	2	19
Moderate	0	0	0	0	0	0	1	11
Total	0	0	2	6*	1	0	3	38**
Urothelium (transitional epithelium) hyperplasia								
Minimal	2	2	1	0	0	0	0	2
Slight	1	2	2	4	0	1	2	26
Moderate	1	0	2	1	0	0	1	11
Total	4	4	5	5	0	1	3	43**
Subacute (chronic/active)/Chronic inflammation								
Minimal	1	1	3	0	1	0	2	12
Slight	1	0	3	1	0	0	1	26
Moderate	0	0	1	1	0	0	1	6
Moderately severe	0	0	0	0	0	0	0	1
Total	2	1	7	2	1	0	4	45**
Lymphoid cell infiltrate/aggregates								
Minimal	6	5	4	9	29	23	24	19
Slight	2	1	0	0	5	5	9	23
Moderate	0	0	0	0	0	0	1	4
Moderately severe	0	0	0	0	0	0	1	0
Total	8	6	4	9	34	28	35	46*
Stromal hyperplasia								
Minimal	0	0	0	1	0	0	0	1
Slight	0	0	0	0	0	0	0	5
Moderate	0	0	0	0	0	0	0	3
Moderately severe	0	0	0	0	0	0	0	2
Total	0	0	0	1	0	0	0	11**
Stromal hypertrophy								
Minimal	0	0	0	0	0	0	0	2
Moderately severe	0	0	0	0	0	0	0	1
Total	0	0	0	0	0	0	0	3
Reticuloendothelial cells: brown pigment accumulation								
Minimal	0	0	0	0	0	0	0	1
Slight	0	0	0	0	0	0	0	6
Total	0	0	0	0	0	0	0	7**

**Statistically different from control, p≤0.05
No microscopic findings in other tissues and organs were observed.

Neoplastic findings consisted of slightly increased incidence of benign submucosal mesenchymal tumours in the urinary bladder female mice at 25 mg/kg bw/day (3/65) compared to controls (0/65).

Incidence of neoplastic changes

Sex	**Males**				**Females**			
DOSAGE (MG/KG BW/DAY)	**0**	**2**	**10**	**25**	**0**	**2**	**10**	**25**
NUMBER OF MICE EXAMINED	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**
Brain (# of tissues examined)	65	22	15	65	65	23	22	65
Malignant Meningioma	0	0	0	0	0	0	0	1
Heart (# of tissues examined)	65	23	15	65	65	22	22	65
Hemangiosarcoma:	0	0	0	1	0	0	1	0
Lungs (# of tissues examined)	65	65	63	65	65	65	65	65
Broncho-Alveolar Adenoma	10	4	8	4	2	1	1	2
Broncho-Alveolar Carcinoma	0	1	1	3	3	1	0	1
Liver	65	65	63	65	65	65	65	65
Hepatocellular Adenoma	6	1	1	6	2	0	0	0
Hemangioma	0	0	1	0	0	0	0	0
Hepatocellular Carcinoma	0	1	2	0	0	0	0	0
Spleen	65	23	16	65	65	23	23	65
Hemangioma	0	0	0	0	1	0	0	0
Hemangiosarcoma	0	1	1	0	1	0	0	0
Stomach	65	65	63	65	64	64	65	65
Forestomach: Hemangioma	0	0	0	0	0	0	0	1
Jejunum	65	59	60	56	62	59	60	64
Carcinoma	0	1	0	0	0	0	0	0
Urinary Bladder	65	63	64	65	65	63	61	65
Urothelial Cell Papilloma	0	0	0	1	0	0	0	0
Submucosal Mesenchymal Tumor	0	0	0	0	0	0	0	**3**
Ovary	0	0	0	0	64	59	54	65
Granulosa/Thecal Cell Tumor	0	0	0	0	0	0	0	0
Luteoma	0	0	0	0	2	0	0	0
Uterus	0	0	0	0	65	51	50	65
Endometrial Stromal Polyp	0	0	0	0	0	0	0	1
Leiomyoma	0	0	0	0	0	0	1	0
Endometrial Stromal Sarcoma	0	0	0	0	1	2	0	3
Leiomyosarcoma	0	0	0	0	1	2	2	2
Rhabdomyosarcoma	0	0	0	0	0	0	1	0
Skin	65	25	19	65	65	26	25	64
Sebaceous Cell Adenoma	1	0	0	0	0	0	0	0
Adrenal Gland	65	22	15	65	65	23	22	65
Cortex: Adenoma	0	0	0	2	0	0	0	0
Cortex: A-Cell Carcinoma	0	0	0	0	0	0	0	1
Thyroid Gland	65	22	14	64	65	23	21	65
C-Cell Adenoma	1	0	0	0	0	0	0	0
Bone	2	1	0	0	0	1	0	0
Osteogenic Sarcoma	1	0	0	0	0	0	0	0
Lymphoreticular	65	65	63	65	65	65	65	65
Malignant Lymphoma	1	2	1	0	3	2	4	2
Histiocytic Sarcoma	0	0	1	1	0	0	0	0
Soft Tissue	2	1	1	3	4	1	2	4
Fibrosarcoma	0	0	0	1	0	0	0	0

The author concluded that administration of DD-92 for 18 months to female Charles River CD-1 mice by oral gavage at a dose of 25 mg/kg/day resulted in a spectrum of lesions attributable to chronic irritation of the urinary bladder which included transitional cell hyperplasia, hyaline change of the lamina propria, and chronic active inflammation. Morphologic evidence of chronic irritation/inflammation of the urinary bladder was present in all mice showing stromal cell hyperplasia/hypertrophy, pigment accumulation, or benign submucosal mesenchymal tumour. The slight increase in benign submucosal mesenchymal tumours in the urinary bladder in the high dose females (3/65 or 4.6%) was not statistically different from the control females (0/65 or 0%). Moreover, the mesenchymal tumours seen in three high-dose females were considered to be a

secondary response to chronic irritation and represent a benign proliferation lesion of minimal or equivocal neoplastic activity. Therefore, the NOEL (no-observable-effect-leve1) for DD-92 when administered to CD-1 mice orally, via gavage, for up to 18 months was 10 mg/kg/day. Administration of DD-92 for 18 months did not result in any carcinogenic effects.

In a two-year dietary chronic toxicity/oncogenicity study in B6C3F1 mice (Anonymous 57, 1995), Telone II soil fumigant (AGR 295646, purity 95.8% 1,3-D (50.7% cis/45.1% trans), a microencapsulated formulation (80:20, starch:sucrose matrix), was administered via the diet (0, 2.5, 25, or 50 mg/kg/day) to sixty male and female mice per dose level for up to 24 months. Control animals were administered placebo sucrose microcapsules. Diet (vehicle for test material) and water were available *ad libitum* during the pre-study and dosing periods. An interim sacrifice group (l0/sex/dose level) was necropsied following a 12-month dosing period. The study was GLP compliant and conducted in accordance with OECD TG 453, with the following deviations; the group sacrificed at 12 months should have contained 20 animals/sex/group instead of the 10 used in this study. Haematology at three and six months should have been performed and 20 animals/sex/group should have been examined instead of 10. Urinalysis and clinical biochemistry were not conducted.

There were no treatment-related increases in mortality in either sex and no treatment related clinical signs of toxicity. The most significant treatment-related effect observed in the study was a dose-related depression in the body weights of male and female mice. The body weights of male mice ingesting 25 or 50 mg/kg/day Telone II were decreased an average of 11-14%relative to controls in a dose-related manner over the majority of the dosing period. Body weight gains of these animals averaged 34-50% less than controls. The body weights of female mice ingesting 25 or 50 mg/kg/day Telone II were decreased an average of 7-9% relative to controls over the majority of the dosing period, and body weight gains averaged 24-28% less than controls. Feed consumption of treated males and females reflected the changes in body weights. Haematology parameters in the control, 2.5, 25, or 50 mg/kg/day male and female mice bled at 12, 18 and 24 months were comparable at each respective time point.

Reflecting the depressed body weights of intermediate and/or high-dose group male and female mice, the absolute and/or relative weights of a number of organs of these animals were statistically identified as being different to controls following 12- and 24-months dosing.

Table 74: Summary of organ weight data from the chronic toxicity/oncogenicity study in B6C3F1 mice (Anonymous 57, 1995)

Dose	0 mg/kg/day		2.5 mg/kg/day		25 mg/kg/day		50 mg/kg/day	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
12 Months								
Final Body weight	39.0	32.2	36.2	31.3	33.2	29.4	28.9	28.6
Heart (Absolute)	0.188	0.160	0.175	0.162	0.164	0.154	0.162*	0.144*
(Relative)	0.482	0.500	0.485	0.520	0.495	0.529	0.563*	0.511
Kidney (Absolute)	0.696	0.491	0.646	0.471	0.650	0.461	0.601*	0.431*
(Relative)	1.787	1.544	1.815	1.512	1.963	1.579	2.084*	1.523
Liver (Absolute)	1.883	1.690	1.732	1.683	1.669$	1.591	1.514$	1.453*
(Relative)	4.845	5.283	4.752	5.393	5.049	5.425	5.248*	5.117
Brain (Absolute)	0.511	0.530	0.492	0.542	0.511	0.539	0.495	0.517
(Relative)	1.320	1.671	1.424	1.738	1.553$	1.847	1.716$	1.838
Testes (Relative)	0.661		0.714		0.784$		0.845$	
24 Months								
Final Body weight	37.2	31.2	36.4	32.1	31.7$	29.8*	30.6$	28.4*
Heart (Absolute)	0.211	0.169	0.213	0.172	0.193*	0.156*	0.191*	0.152*
(Relative)	0.571	0.543	0.590	0.537	0.608*	0.524	0.624*	0.537
Kidney (Absolute)	0.792	0.494	0.782	0.497	0.761	0.450*	0.720*	0.441*
(Relative)	2.141	1.586	2.159	1.552	2.401*	1.530	2.361*	1.557
Liver (Absolute)	2.210	1.854	2.272	1.750	2.126$	1.682$	1.824$	1.660$
(Relative)	5.976	5.904	6.274	5.443	6.682$	5.603	5.961	5.796
Brain (Absolute)	0.518	0.514	0.518	0.514	0.515	0.511	0.521	0.509
(Relative)	1.410	1.656	1.439	1.612	1.632$	1.726	1.708$	1.799*
Testes (Relative)	0.634		0.645		0.741*		0.777*	

* Statistically different from control mean by Dunnett's test, alpha = 0.05.

$ Statistically different from control mean by Wilcoxon's test, alpha = 0.05.

Histopathologic Observations--12-Months--Males (Liver)

Sex	Males			
Dose (mg/kg/day)	0	2.5	25	50
Number of Mice Examined	50	50	50	50
Liver (# of Tissues Examined)	**10**	**10**	**10**	**10**
Within normal limits	1	3	3	1
Aggregate(s) of reticuloendothelial cells: -very slight	7	5	6	5
Atrophy secondary to inanition, hepatocellular, diffuse:	0	1	0	1
Decreased size, hepatocellular, diffuse: -slight	0	0	0	6
Focus(I) of altered cells, hepatocellular, focal: -very slight	2	1	0	0
Necrosis - individual cell(s), hepatocellular, focal: -slight	0	0	0	0
Vacuolation consistent with fatty change, hepatocellular, diffuse:	0	1	0	0
Necrosis with accompanying inflammation, hepatocellular, focal: -slight	0	0	1	0
Necrosis with accompanying inflammation, hepatocellular, multifocal: -slight	0	0	0	1
Necrosis with accompanying inflammation, hepatocellular, focal or multifocal: -slight	0	0	1	1
Adenoma, hepatocellular, benign, primary:	0	0	1	0

Incidences of lung tumours

LUNGS (NO. OF TISSUES EXAMINED)	50	50	50	50		50	50	50	50
ADENOCARCINOMA, BRONCHIOLOALVEOLAR, MALIGNANT, PRIMARY, NO METASTASIS:	2	4	1	0		0	0	0	0
ADENOCARCINOMA, BRONCHIOLOALVEOLAR, MALIGNANT, PRIMARY, NO METASTASIS: (TWO)	1	0	0	0		0	0	0	0
ADENOCARCINOMA, BRONCHIOLOALVEOLAR, MALIGNANT, PRIMARY, METASTASIS:	0	0	0	0		0	1	0	0
ADENOCARCINOMA, BRONCHIOLOALVEOLAR, MALIGNANT, PRIMARY, METASTASIS OR NO METASTASIS: (ONE OR TWO)	3	4	1	0	**	0	1	0	0
ADENOCARCINOMA, (LACRIMAL GLAND), MALIGNANT, SECONDARY:	0	0	0	0		0	0	1	0
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY:	4	9	9	9		3	8	4	5
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY: (TWO)	0	1	0	2		0	0	0	0
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY: (THREE)	1	0	0	0		0	0	0	0
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY: (ONE OR TWO OR THREE)	5	10	9	11	**	3	8	4	5
CARCINOMA, (KIDNEY), MALIGNANT, SECONDARY:	0	0	0	1		0	0	0	1
CARCINOMA, (LIVER), MALIGNANT, SECONDARY:	3	0	0	1		0	0	0	0
TOTAL ANIMALS WITH ADENOMA AND/OR ADENOCARCINOMA, BRONCHIOLOALVEOLAR:	6	13	10	11		3	9	4	5

Relevant HCD for B6C3F1 Lung Tumors								
Year	Number of Mice	Exposure Route	Adenoma		Carcinoma		Combined adenoma and carcinoma	
			Male	Female	Male	Female	Male	Female
1981	96	Dietary	6 (6.3%)	4 (4.2%)	2 (2.1%)	0	ND	ND
1983	86	Dietary	7 (8.1%)	8 (9.3%)	2 (2.3%)	0	7 (8.1%)	8 (9.3%)
1984	50	Dietary	12 (24%)	3 (6%)	2(4%)	2 (4%)	12 (24%)	3 (6%)
1984	50	Inhalation	10 (20%)	3 (6%)	2 (4%)	1 (2%)	10 (20%)	3 (6%)
1985	50	Dietary	7 (14%)	7 (14%)	2 (4%)	1 (2%)	7 (14%)	7 (14%)
1986	50	Dietary	5 (10%)	3 (6%)	3 (6%)	2 (4%)	5 (10%)	3 (6%)
1990	50	Dietary	12 (24%)	2 (4%)	3 (6%)	1 (2%)	12 (24%)	2 (4%)
1990	50	Dietary	12 (24%)	2 (4%)	2 (4%)	2 (4%)	14 (28%)	4 (8%)
1990	50	Dietary	10 (20%)	2 (4%)	2 (4%)	0	12 (24%)	2 (4%)
1991	50	Dietary	12 (24%)	4 (8%)	1 (2%)	0	12 (24%)	4 (8%)
1992	50	Dietary	12 (24%)	4 (8%)	2 (4%)	1(2%)	12 (24%)	4 (8%)
1995	50 (M)/48 (F)	Dietary	14 (28%)	5 (10.4%)	6 (12%)	2 (4.2%)	14 (28%)	5 (10.4%)
1995	50	Dietary	18 (36%)	ND	1 (2%)	NA	19 (38%)	ND
1995	50	Dietary	9 (18%)	2 (4%)	0	2 (4%)	ND	ND
1997	50	Dietary	15 (30%)	7 (14%)	1 (2%)	0	15 (30%)	7 (14%)
1997	50	Dietary	14 (28%)	4 (8%)	3 (6%)	1 (2%)	14 (28%)	4 (8%)
1998	50	Inhalation	9 (18%)	8 (16%)	5 (10%)	0	9 (18%)	8 (16%)

ND= no data

Most organ weight changes lacked any histopathological correlate. However, hepatocytes of high-dose group males following 12-months dosing were observed to be slightly decreased in size with smaller cytoplasmic volume than controls. This change was consistent with decreased cellular glycogen content and corresponded to a statistically identified decrease in absolute liver weights of high-dose group males. A similar histologic change was not noted following 24 months of dosing. No treatment-related increased incidence of tumours was observed in mice ingesting Telone II. The NOAEL for systemic toxicity was 2.5 mg/kg bw/day Telone II for male and female mice. No increased incidence of tumours was observed in mice ingesting Telone II for two years. The oncogenic NOAEL was 50 mg/kg bw/day.

NTP, 1985 (and references therein)

Toxicology and carcinogenesis ingestion studies of Telone II® (88%–90% 1,3-dichloropropene, 2.5% 1,2-dichloropropane, 1.5% trichloropropene isomer, and 1% epichlorohydrin) were conducted by administering the commercial-grade formulation in corn oil by gavage to groups of 50 male and 50 female B6C3F1 mice at doses of 0, 50, or 100 mg/kg, three times weekly for 104 weeks. At study termination, there were no toxicologically significant changes in body weight. However, 25 vehicle control mice died from myocarditis during weeks 48 –51. Survival of female mice was statistically lower in the 100 mg/kg group.

Nonneoplastic lesions. Three types of nonneoplastic changes were observed in mice. Epithelial hyperplasia of the forestomach was statistically significant for females at 100 mg/kg but not for males in any treated group. The incidence of transitional epithelial hyperplasia of the urinary bladder (see Table 75) was also observed with statistical significance in both sexes at 50 mg/kg and 100 mg/kg. Such lesions in the urinary bladder have also been noted in inhalation studies. Hyperplasia of the transitional epithelium of the urinary bladder was observed in female mice exposed to ≥409 mg/m^3 technical-grade dichloropropene for 13 weeks (Stott et al., 1988) and to 90.8 mg/m^3 for 2 years (Lomax et al., 1989). In the chronic study (Lomax et al., 1989), male mice were affected at 272 mg/m^3 technical-grade dichloropropene. The third nonneoplastic change found in mice (NTP, 1985) was an increased incidence of hydronephrosis exhibited by female mice in the 100 mg/kg group. For mice, the LOAEL is 50 mg/kg, based on epithelial hyperplasia in the urinary bladder. Averaging the exposure over 7 days yields a LOAEL of 21.4 mg/kg/day. There is no NOAEL for mice.

Table 76: Incidence[a] of selected cancer and noncancer effects in mice from NTP (1985) 2-year study

	Males			Females		
Lesion	0 mg/kg/day	50 mg/kg/day	100 mg/kg/day	0 mg/kg/day	50 mg/kg/day	100 mg/kg/day
Basal cell or epithelial hyperplasia of stomach	0/52	0/50	4/50	1/50	1/50	21/50
Squamous cell papilloma/carcinoma of forestomach	0/37	2/47	3/49	0/50	1/50	4/47
Bronchioalveolar adenoma/carcinoma	1/22	13/40	12/44	2/50	4/50	8/47
Urinary bladder epithelial hyperplasia	0/50	9/50	18/50	2/50	15/50	19/47
Urinary bladder transitional cell carcinoma	0/50	0/50	2/50	0/50	8/50	21/47

[a] 50 mice started in each group. For tumor incidence, mice that died before the first tumor appeared were omitted from the number at risk

Neoplastic lesions In mice, the most toxicologically significant neoplastic finding was a dose-related statistically significant increase in the incidence of transitional cell carcinoma of the urinary bladder in females in both 50 and 100 mg/kg dose groups. Two males in the 100 mg/kg group also developed transitional cell carcinoma of the bladder, but the incidence was not statistically significant. Urinary bladder carcinoma was not observed in the 2-year feeding study of Redmond et al. (1995).

An increase in the incidence of bronchioalveolar adenomas in the lung was statistically significant in female mice at 100 mg/kg. One carcinoma was found in the 50 mg/kg group but not in the 100 mg/kg group. In male mice, a statistically significant increase in the incidence of bronchioalveolar adenomas was observed at 50 mg/kg but not at 100 mg/kg. Two additional males in the 50 mg/kg group and three additional males in the 100 mg/kg group were diagnosed with bronchioalveolar carcinoma. Thus, the combined incidences of lung adenomas and carcinomas in male mice reached statistical significance for both 50 and 100 mg/kg groups. A significant increase in the incidence of bronchioalveolar adenomas was also observed in male mice exposed to 272 mg/m^3 technical-grade dichloropropene by inhalation for 24 months (Lomax et al., 1989).

Forestomach tumors were also observed in mice. The incidences for both males and females were statistically significant at 100 mg/kg.

With regard to the mouse studies, NTP concluded that the male mouse study was inadequate for investigation of carcinogenicity because of the greatly reduced survival in the vehicle control group. In females, however, there was clear evidence of carcinogenicity, based on the increased incidence of transitional cell carcinoma of the urinary bladder, a very rare form of rodent cancer. Supporting evidence for carcinogenicity of Telone II® in female mice included the increased incidences of alveolar/bronchiolar adenomas of the lung and combined squamous cell papillomas and/or carcinomas of the forestomach (not statistically significant) at the highest dose, 100 mg/kg. Chronic toxicity of Telone II® was evidenced by hyperplasia of the forestomach in both sexes of mice, and epithelial hyperplasia of the urinary bladder in male and female mice. Based on the serial-sacrifice (ancillary) study, development of both hyperplasia and carcinogenicity of the forestomach in rats was dependent on exposure duration.

On the basis of urinary bladder and lung neoplasms in mice, the LOAEL for cancer in the NTP (1985) study is 21.4 mg/kg (50 mg/kg/day × 3 days/7 days) and there is no NOAEL for mice.

Carcinogenicity: inhalation

Rat:

In a 2-year combined chronic toxicity and carcinogenicity study in male and female Fischer 344 albino rats, interim sacrifice groups (10 animals/sex/exposure concentration/interim sacrifice group) were exposed to 1,3-D vapours for 6 hrs/ day, 5 days/week for either 6 months or 12 months as part of a larger 2-year chronic toxicity-oncogenicity study having 70 rats/sex/exposure concentration. The target exposure concentrations tested were 0, 5, 20 and 60 ppm (0, 22.7, 90.8 and 272 mg/m3, respectively), equivalent to achieved doses of 0, 4.43, 17.74 or 53.22 mg/kg bw/day respectively.

In the interim study report (Anonymous 55, 1985), covering the 6- and 12-month exposure periods, no treatment-related effects upon the general appearance and demeanour of male or female rats of either interim sacrifice group were observed and all animals survived until their scheduled sacrifice time. At 60 ppm, males but not females showed a statistically significant decrease in body weight at 6 months vs control rats. However, when data from all animals of the different sacrifice groups (6, 12 and 24 months) were combined and analysed, a small depression in the body weight of the high exposure level group (3-4%) was observed vs controls in males and females. No treatment-related effects upon haematological or urinalysis parameters measured were observed in either the 6- or 12-month interim sacrifice groups of rats. Statistically significant decreases in several clinical chemistry parameters in top exposure group female rats of the 6-month (serum BUN, total proteins and albumins) and 12-month (serum GPT) interim sacrifice groups were not considered to be related to 1.3-D exposure.

Only one palpable mass was observed in either interim sacrifice group of rats. This small (5mm diameter) mass occurred near the external genitalia of a male rat exposed to 60 ppm 1,3-D but had resolved prior to sacrifice of the animal. There were no treatment-related effects of 1,3-D exposure upon brain, heart, kidneys, liver and testes weights in either the 6- or 12-month interim sacrifice groups of rats. Likewise, there were no

treatment related observable pathologic changes or histopathological changes noted in either sex of interim sacrifice groups of rats.

It was noted that lesions of the nasal mucosa which had previously been reported to occur at a low incidence in male rats exposed to 30 ppm 1,3-D, and all rats exposed to 90 ppm 1,3-D, for 13-weeks (Anonymous 73, 1984) were not observed in the present study in rats exposed to 60 ppm 1,3-D for up to 12 months, suggesting that this lesion is not progressive in rats with increasing time of exposure and may even resolve with time.

In the final study report for the 2-year inhalation chronic toxicity and carcinogenicity study in male and female Fischer 344 albino rats (Anonymous 58, 1987), groups of 70 male and 70 female Fischer 344 rats were exposed by inhalation to vapors of TELONE II soil fumigant for 6 hours/day, 5 day/week for up to 24 months (total of 509 days of exposure) at targeted concentrations of 0, 5, 20, or 60 ppm (0, 22.7, 90.8, or 272.4mg/m^3). No clinical signs of toxicity were observed in exposed animals throughout the study. There were no increases in palpable masses due to exposure, and no significant differences in survival were observed between any groups of either sex.

Table 77: Summary of survival (%) for the 2-year inhalation chronic toxicity and carcinogenicity study in Fischer 344 albino rats (Anonymous 58, 1987)

Dose (ppm)	**0**		**5**		**2**		**60**	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
% survival	46	60	56	52	60	76	56	72

Mean body weights of both males and females exposed to 60 ppm were statistically decreased from mean control values. For high exposure male rats, mean body weights were decreased approximately 5% from control male rats on test days 13- 425, but were similar to control rats throughout the remainder of the study. High exposure female rats had a similar mean body weight decrease of approximately 5% on test days 6-327 and were similar to control values throughout the remainder of the study. There was no effect of treatment on haematology parameters or urinalysis. There were no statistically significant different in clinical chemistry parameters in treated males. Female rats exposed to 60 ppm had statistically significantly lower mean total protein and albumin concentrations; however, these subtle variations were not considered to be toxicologically significant. There was no effect of treatment on absolute and adjusted organ weights, and no gross pathologic observations related to 1,3-D exposure.

Histopathologic examinations showed statistically significant effects in nasal tissues of male and female rats exposed to 60 ppm 1,3-D. These were unilateral or bilateral decreased thickness of olfactory epithelium, unilateral or bilateral erosions of olfactory epithelium, and unilateral or bilateral submucosal fibrosis (underlying olfactory mucosa). The prevalence of these changes by anatomic region of the nasal cavity (levels 1-4) is shown in the following table.

Table 78: Summary of histopathological effects in the nasal tissue of male and female rats exposed to 60 ppm 1,3-D (Anonymous 58, 1987)

Microscopic Change	Number examined	Number affected	Overall incidence (%)	Anatomic Level [a]			
				1	2	3	4
Males 60 ppm							
Decreased thickness olfactory epithelium	50	20	40	0	17	15	10
Erosions of olfactory epithelium	50	15	30	0	12	14	10
Submucosal Fibrosis	50	6	12	0	4	5	6
Females 60 ppm							
Decreased thickness olfactory epithelium	49	15	31	0	12	8	5
Erosions of olfactory epithelium	49	6	12	0	5	4	4
Submucosal Fibrosis	49	2 [b]	4	0	0	1	1

[a] Any given animal may have 1 or more levels affected. Level l = most, anterior, obtained just posterior to the incisor teeth; Level 2 = obtained at the incisive papilla; Level 3 = obtained at level of second palatal ridge; Level 4 = most posterior, obtained at level of first upper molar teeth.

[b] Not statistically significant.

Decreased thickness of olfactory epithelium at levels 2, 3, and 4 was the most prevalent microscopic change in both male and female rats. This change usually occurred in olfactory epithelium adjacent to the dorsal meatus. Erosions of olfactory epithelium also occurred in levels 2, 3, and 4 of male and female rats, adjacent to the dorsal meatus, and multifocally on ecto- and endoturbinates. Fibrosis of submucosal tissues underlying eroded olfactory epithelium was observed at levels 2, 3, and 4 in male rats and occurred primarily on ecto- and/or endoturbinates. A variety of other inflammatory, degenerative, and/or hyperplastic, microscopic changes occurred in portions of the respiratory epithelium from some animals in all exposure groups and controls.

There were no statistically identified increases in any tumour incidence in rats exposed to 1,3-D. Male rats exposed to 60 ppm had a slight increase in benign subcutaneous fibromas (10% incidence versus 6% incidence in controls). This incidence was unchanged when subcutaneous fibromas were combined with mammary gland fibromas. However, the incidence of fibroma observed in this study was normal for animals of this age and strain at this laboratory as shown by the historical control data.

Table 79: Incidence of selected fibromas in male rats exposed to 1,3-D for 2 years (Anonymous 58, 1987)

Exposure Concentration (ppm)	**0**	**5**	**20**	**60**
Skin and subcutis				
Fibroma, subcutaneous, benign, primary	3	3	3	5
Mammary gland				
Fibroma, benign, primary	0	1	0	0
Combined Total	**3**	**4**	**3**	**5**

Historical laboratory data from previous 2-year studies using Fischer 344 rats revealed a combined incidence of 9.5% with a range of 2-22% for benign fibromas in control male rats (all routes of exposure). Taken into account inhalation studies, HCD are available for 2 studies with values of 10 and 22 % for benign fibromas in control male rats.

Table 80: Historical Control Fibromas in F-344 rats

Study	A	B		C		D	E	F	G	H	Overall incidence
Route of exposure	Diet	Inhalation		Diet		Drinking water	Diet	Diet	Inhalation	Diet	
Report date	1982	1983		1983		1984	1984	1986	1986	1987	
Number of control males	86	60	60	50	50	60	50	50	50	50	Total = 566
MALES											
Mammary fibroma											
One	1	0	0	3	1	3	6	6	0	0	3%
Two	0	0	0	0	0	0	1	0	0	0	0.2%
Total	1	0	0	3	1	3	7	6	0	0	3.7%
Subcutaneous fibroma											
One	1	6	8	3	1	0	1	0	11	1	5.7%
Two	0	0	1	0	0	0	0	0	0	0	0.2%
total	1	6	9	3	1	0	1	0	11	1	5.8%
Total number with fibroma(s) subcutaneous or mammary	2	6	9	6	2	3	8	6	11	1	9.5%
Total in Percent	2%	10%	15%	12%	4%	5%	16%	12%	22%	2%	Range 2-22%

Exposure to vapours of 1,3-D for 2 year did not cause treatment related tumours in either male or female rats. The chronic NOAEC was 20 ppm based on body weight decrease and olfactory epithelial changes at 60 ppm. The carcinogenic NOAEC is 60 ppm in the absence of tumours.

Mice:

In the two-year inhalation chronic toxicity/oncogenicity study with Telone II soil fumigant in mice- Interim report; 6- and 12-month exposures (Anonymous 55, 1985), groups of 70 males and 70 female B6C3F1 mice were exposed to Telone II (Lot No. TB831213-4: 92.1% of 1,3-D (cis 49.5% and trans 42.6%); l,2-dichloropropane, 0.7%; 1,3-dichloropropane, 1.8%; 1-chlorohexane, 1.1% and the remainder mixed isomers of chlorohexene, chlorohexane and trichlopropene) vapors for 6 hours/day, 5 days/week to targeted exposure concentrations of 0, 5, 20, or 60 ppm (corresponding to 0, 22.7, 90.8 or 272.4 mg/m3, respectively or equivalent to 0, 7.69, 30.75 and 92.24 mg/kg bw/day, respectively). The study was GLP compliant and conducted in accordance with OECD Guideline No. 453 with the following deviations; there were only 10 (as opposed to 20) mice/sex/dose in the satellite groups for 6 and 12-month sacrifices; food consumption not conducted; ophthalmology not conducted; haematological examination was not conducted at 3 months; urinalysis was not conducted.

Ten mice/sex/exposure level were predesignated for 6 and 12 months of exposure. The remaining 50 mice/sex/group level were designated for the oncogenicity portion of the study and were scheduled for termination at 24 months.

6-Month exposure

At 6 months no changes in body weight, haematological examinations and clinical chemistry were observed.

Decreased liver and kidney weights were present in male mice from the 60 ppm exposure group. These weight changes were histologically associated with a decrease in the degree of vacuolation normally observed in hepatocytes and renal epithelial cells of the proximal convoluted tubules. Although equivocal decreases in liver and kidney weights were present in males from the 20 ppm exposure group, similar histopathologic changes were not observed in these males or females at any level. Histological changes were also present in the urinary bladders of 1/10 male and 4/10 female mice from the 60 ppm exposure group and were characterised by a moderate hyperplasia of the transitional epithelium. This hyperplastic reaction was occasionally accompanied by an inflammatory reaction in the lamina propria of the urinary bladder. Neither hyperplasia nor inflammatory lesions occurred in male or female mice from the lower exposure groups.

Focal hyperplasia and hypertrophy of the respiratory epithelium was histologically observed in the nasal turbinates of male and female mice exposed to 60 ppm and males exposed to 20 ppm Telone II. This lesion was very slight to slight in degree, was located in only the most anterior section, and was most prominent in males from the 60 ppm exposure group.

Table 81: Summary of organ weight data for male mice following 6-months exposure to Telome II (Anonymous 55, 1985)

Concentration	Body weight	Kidneys		Liver	
(ppm)	(g)	(g)	(g/100g bw)	(g)	(g/100 g bw)
0	30.5	0.561	1.838	1.632	5.345
5	31.1	0.551	1.770	1.634	5.244
20	29.7	0.522*	1.759	1.544	5.194
60	29.9	0.478*	1.598*	1.425*	4.760*

* Statistically different from control mean by Dunnett's test, alpha = 0.05.

Table 80: Histopathologic Observations (6-Month-Nasal Tissue, Urinary Bladder, Liver, Kidney) (Anonymous 55, 1985)

Sex	Males				Females			
Dose (mg/kg bw/day)	**0**	**5**	**20**	**60**	**0**	**5**	**20**	**60**
Number of mice examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Nasal Tissues (# of Tissues Examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Inflammation – acute -very slight	0	0	1	0	0	0	0	1
Hyperplasia and hypertrophy, respiratory epithelium, focal: -very slight	1	0	3	2	0	0	0	7
-slight	0	0	0	8	0	0	0	0
Urinary Bladder (# of Tissues Examined)	**10**	**10**	**10**	**9**	**10**	**10**	**10**	**10**
Aggregate(s) of mononuclear (predominantly lymphoid) cells-very slight	9	7	8	6	7	7	7	4
-slight	0	1	2	2	2	2	2	4
Hyperplasia, epithelial cells: -moderate	0	0	0	1	0	0	0	4
Inflammation – subacute to chronic: -very slight	0	0	0	0	0	0	0	1

-slight	0	0	0	1	0	0	0	0
-moderate	0	0	0	0	0	0	0	1
Liver (# of Tissues Examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Vacuolation – decreased, hepatocellular: -slight	1	0	0	7	0	0	0	0
Kidneys (# of Tissues Examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Vacuolation – decreased, convoluted tubule - proximal: -slight	4	1	2	9	0	0	0	0

12-Month exposure

The only in-life observations interpreted to be treatment-related were slight decreases in body weight in male and female mice exposed to 60 ppm of Telone II during the last 6 months of exposure.

Hyperplastic and hypertrophic lesions involving the respiratory epithelium of the nasal turbinates were present in male and female mice exposed to 60 ppm and males exposed to 20 ppm of Telone II. These changes were similar to those noted after 6 months of exposure.

Male mice exposed to 60 ppm of Telone II had decreased liver and kidney weights which were again associated with a decrease in the degree of vacuolation normally observed in hepatocytes and renal epithelial cells. This decrease in vacuolation in hepatocytes was interpreted to reflect a decrease in hepatic glycogen. A slight decrease in the relative liver weight was again noted in males from the 20 ppm exposure group, however, this was not associated with a histopathologic change. Female mice did not have similar liver or kidney changes at any level.

The mucosa of the urinary bladder of a few female mice exposed to Telone II was thickened. Hyperplasia of the transitional epithelium of the urinary bladder, similar to that noted at 6 months, was histologically observed in the majority of females exposed to 60 ppm and in 1/10 females in the 20 ppm exposure group. This hyperplastic reaction was characterised by a simple thickening of the epithelium and involved a variable amount of the mucosa, but primarily occurred in the area of the anterior ventral wall. Focal papillary hyperplasia, epithelial pigmentation, and vacuolization were also occasionally present. In addition to the epithelial changes, the lamina propria underlying the hyperplastic epithelium frequently contained inflammatory cells and prominent fibroblasts, connective tissue and vasculature.

Table 81: Organ Weights 12-Months—Males (Anonymous 55, 1985)

Concentration	Body weight	Kidneys		Liver	
(ppm)	(g)	(g)	(g/100g bw)	(g)	(g/100 g bw)
0	31.1	0.592	1.899	1.681	5.390
5	31.1	0.604	1.941	1.596	5.133
20	30.7	0.593	1.932	1.534	4.989*
60	30.0	0.513*	1.710*	1.381*	4.599*

* Statistically different from control mean by Dunnett's test, alpha = 0.05.

Table 82: Histopathologic Observations (12-Month-Nasal Tissue, Urinary Bladder, Liver, Kidney) (Anonymous 55, 1985)

Sex	Males				Females			
Dose (mg/kg bw/day)	**0**	**5**	**20**	**60**	**0**	**5**	**20**	**60**
Number of mice examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Nasal Tissues (# of Tissues Examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Decreased thickness, olfactory epithelium, focal: -very slight	0	0	0	0	1	0	0	0
Decreased thickness, olfactory epithelium, multifocal: -very slight	0	0	0	0	0	0	0	2
Inflammation – acute -very slight	0	1	0	0	1	1	0	1
Inflammation – subacute to chronic: - very slight	0	0	0	0	0	0	1	1
Hyperplasia and hypertrophy, respiratory epithelium, focal: -very slight	1	0	7	3	0	0	0	8
-slight	0	0	0	7	0	0	0	0
Urinary Bladder (# of Tissues Examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Aggregate(s) of mononuclear (predominantly lymphoid) cells-very slight	5	7	8	7	8	6	7	3
-slight	0	0	0	0	2	4	3	2
Hyperplasia, epithelial cells: -slight	0	0	0	0	0	0	1	5
-moderate	0	0	0	0	0	0	0	4

Sex		Males				Females			
Dose (mg/kg bw/day)		**0**	**5**	**20**	**60**	**0**	**5**	**20**	**60**
Number of mice examined		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Inflammation – subacute to chronic:	-very slight	0	0	0	0	0	0	0	1
	-slight	0	0	0	0	0	0	0	3
Liver (# of Tissues Examined)		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Vacuolation – decreased, hepatocellular:	-slight	0	0	0	5	0	0	0	0
	-severe	1	0	1	0	0	0	0	0
Kidneys (# of Tissues Examined)		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Within normal limits:		4	4	5	0	7	9	7	8
Vacuolation – decreased, convoluted tubule - proximal:	-slight	1	0	0	4	0	0	0	0
	-moderate	0	0	0	5	0	0	0	0

Treatment-related effects were observed in male and female mice exposed at 20 or 60 ppm and were similar after 6 and 12 months of exposure. Hyperplasia and hypertrophy of the respiratory epithelium of the nasal turbinates were histologically observed in male and female mice at 60 ppm and males at 20 ppm. Decreased liver and kidney weights which were accompanied by histological evidence of a decrease in vacuolation of hepatocytes and renal epithelial cells were present in males from the 60 ppm exposure group. The most important treatment-related effect was observed in the urinary bladder of female mice exposed to 60 ppm of Telone II for 6 and 12 months and consisted of a slight to moderate hyperplasia of the transitional epithelium.

Based upon the organ weight and histopathologic treatment-related changes, no-observed-adverse effect level (NOAEL) for Telone II vapors in this study was 5 ppm, the lowest dose level.

In the two-year inhalation chronic toxicity/oncogenicity study with Telone II soil fumigant in mice- final report (Anonymous 56, 1987), data for the remaining 50 mice/sex/group level, which were designated for the 24 months chronic toxicity-oncogenicity study are reported.

There was no evidence of an increased mortality of mice exposed to vapors of Telone II soil fumigant relative to control animals. No dose-related increase in total palpable masses was noted in exposure groups of animals relative to controls.

The body weights of male mice exposed to 60 ppm vapors of TELONE II soil fumigant were statistically depressed 3 to 9% relative to those of control males. This difference was evident following the first week of exposure, sporadically during the first 4 months of exposure, and for nearly all the remainder of the study. A similar depression in body weights (2-11%) was also noted to occur in high exposure group females; however, apart from the first week of study, statistically significant lower body weights were only recorded following the first 5 months of the study.

A number of alterations in several clinical chemistry and hematologic parameters were observed in male mice exposed to 60 ppm vapors. These changes included; a decrease in RBC numbers and HCT; increased serum urea nitrogen (UN) content and alkaline phosphatase (AP) activity; and decreased serum GLOB content. However, with the possible exception of serum UN, these small changes were not considered to reflect an adverse effect of treatment upon the health of the animals and probably represent normal variations in these measurements.

Table 83: Haematology & Clinical Chemistries – Males following 2-years exposure to Telone II (Anonymous 56, 1987)

Conc. (ppm)	RBC x10^6/CU MM	HCT %	BUN mg/dL	AP mU/mL	Globulin g/dL
0	9.31	37.5	22	48	2.6
5	9.16	36.8	24	51	2.7
20	9.04	36.7	22	51	2.5
60	8.79$	35.8*	26*	53*	2.3$

* Statistically different from control mean by Dunnett's test, alpha = 0.05.
$ Statistically different from control mean by Wilcoxon's test, alpha = 0.05.
Red blood cell (RBC), haematocrit (HCT), blood urea nitrogen (BUN), alkaline phosphatase (AP),

The mean terminal body weight of male mice at 60 ppm was approximately 4% lower than that of control mice. A similar, though not statistically significant, difference in the body weight females at 60 ppm was also observed.

The mean relative and/or absolute weights of heart, kidney and liver from males at 60 ppm were slightly lower (10-15%) than those of control. In addition, a small, yet statistically significant increase (3%) in the relative brain weights of these mice was noted. In females, only relatively minor decreases were observed in the mean absolute brain and heart weights at 60 ppm. The relatively small nature of these organ weight differences and the fact that, apart from the kidneys, they were not associated with treatment-related histopathologic changes in these tissues (see below), suggests that these were not toxicologically significant and in most instances simply reflected the depressed body weights of these mice and/or normal biological variability.

Table 84: Organ Weights--Males following 2-years exposure to Telone II (Anonymous 56, 1987)

Dose (ppm)	Body weight	Brain	Heart		Kidneys		Liver
(ppm)	(g)	(g/100 g bw)	(g)	(g/100 g bw)	(g)	(g/100 g bw)	(g)
0	28.9	1.609	0.159	0.550	0.599	2.072	1.530
5	29.6	1.584	0.159	0.539	0.636$	2.515	1.560
20	28.8	1.616	0.153	0.530	0.580	2.012	1.519
60	27.6*	1.662*	0.137*	0.497*	0.507$	1.814$	1.357$

* Statistically different from control mean by Dunnett's test, alpha = 0.05.
$ Statistically different from control mean by Wilcoxon's test, alpha = 0.05.

At gross examination there was an increased incidence (14/50 mice) of one or more lung masses/animal observed in males at 60 ppm relative to control (5/50). The urinary bladder mucosal surface appeared roughened at low power microscopic (dissecting stereomicroscope) examination of many female mice exposed to 20 or 60 ppm and some males exposed to 60 ppm. Masses were noted in one female and one male from the 20 and 5 ppm exposure groups, respectively.

Table 85: Summary of gross pathologic observations (lungs and urinary bladder) (Anonymous 56, 1987)

Sex	Male				Female			
Exposure concentration (ppm)	**0**	**5**	**20**	**60**	**0**	**5**	**20**	**60**
Number of mice examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Lungs								
Mass/nodule	5	3	8	13	2	3	3	2
Mass/nodule (two)	2	0	1	1	0	0	0	0
Urinary Bladder								
Within normal limits	50	49	50	50	50	50	50	50
Focus - dark, wall	0	1	0	0	0	0	0	0
Urinary Bladder (Using dissecting Microscope on Fixed Distended Urinary Bladders								
Roughened, irregular and opaque surface -slight	0	1	0	2	3	4	7	14
Roughened, irregular and opaque surface -moderate	0	0	0	3	0	1	11	14
Roughened, irregular and opaque surface -marked	0	0	0	1	0	0	2	2
Mass or nodule	0	1	0	0	0	0	1	0

Histopathology Non-neoplastic: Microscopic examination of tissues revealed statistically significant increases in treatment-related effects in the urinary bladder and nasal mucosa of both sexes of mice, non-glandular portion of the stomach and kidneys of males, and the livers of females exposed to 20 ppm or 60 ppm vapors or both.

Urinary bladder effects consisted of an exposure-related increase in the occurrence and severity of hyperplasia of the transitional epithelium of the urinary bladders of nearly all males and females at 60 ppm and in several males and females at 20 ppm. In most cases this lesion was characterized by a diffuse, smooth surfaced thickening of the epithelium (simple hyperplasia). In general, more severe effects were noted in females than

males with 21/45 females and only 3/47 males having pronounced hyperplastic changes. An inflammatory reaction was often associated with the hyperplastic response of the urinary bladder mucosa in female mice. Aggregates of lymphoid cells were noted in exposed mice (chronic inflammation).

Table 86: Incidence of various types and grades of hyperplasia (urinary bladder) (Anonymous 56, 1987)

Sex	**Male**				**Female**			
Exposure concentration (ppm)	**0**	**5**	**20**	**60**	**0**	**5**	**20**	**60**
Number of mice examined	**47**	**48**	**48**	**47**	**47**	**46**	**48**	**46**
Hyperplasia (simple) mucosa								
Very slight	4	7	7	16	1	3	13	5
Slight	0	0	3	18	0	1	6	18
Moderate	0	0	0	2	0	0	0	19
Hyperplasia-nodular, mucosa								
Slight	0	0	1	0	0	0	0	0
Moderate	0	0	0	1	0	0	2	2

Nasal mucosa effects consisted of hypertrophy and hyperplasia of the respiratory epithelium and degeneration of the olfactory epithelium in nearly all males and females at 60 ppm. Hyperplasia of the respiratory epithelium was also noted in a majority of females at 20 ppm. Respiratory epithelial lesions were characterized by their bilateral occurrence in a symmetrical pattern at the rostral aspects of the nasal cavity. The normally thin layer of one or two cuboidal cells of these areas appeared thickened and pseudostratified in affected mice.

Table 87: Incidence of lesions of the nasal mucosa (Anonymous 56, 1987)

Sex	**Male**				**Female**			
Exposure concentration (ppm)	**0**	**5**	**20**	**60**	**0**	**5**	**20**	**60**
Number of mice examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Respiratory Epithelium								
Hypertrophy and hyperplasia, respiratory mucosa bilateral								
Very slight	5	1	4	38	4	4	28	39
Slight	0	0	0	10	0	0	0	10
Olfactory Epithelium								
Degeneration, olfactory epithelium bilateral								
Very slight	1	0	1	32	0	0	1	29
Slight	0	0	0	16	0	0	0	16

Treatment-related effects in the non-glandular portion of the stomach consisted of hyperplasia of the epithelium which was statistically significant in males at 6- ppm only. This lesion was characterized by a thickening of the stratified squamous mucosa, accentuation of the rete pegs and hyperkeratosis of the stratified squamous mucosa. Mononuclear inflammatory cells were often noted in the submucosa and small foci of granulocytic cells or ulcers were also occasionally seen in the thickened portions of the mucosa.

Table 88: Incidence of hyperplasia of the epithelium of the non-glandular portion of the stomach (Anonymous 56, 1987)

Sex	**Male**				**Female**			
Exposure concentration (ppm)	**0**	**5**	**20**	**60**	**0**	**5**	**20**	**60**
Number of mice examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Hyperplasia, often accompanied by chronic inflammation focal or multifocal	0	3	1	8	0	0	0	2

Decreased vacuolation of the renal tubular epithelial cells was observed in over half of the males (29/50 vs 9/50 in controls) and the liver cells in roughly half the female mice (24/50 vs. 10/50 in controls) at 60 ppm. Renal and hepatic changes were noted in male mice following 6- and 12-months exposure and were also associated with decreased organ weights (Anonymous 55, 1985). These changes were consistent with a relative decrease in cellular lipid and glycogen content of renal tubular epithelial cells and hepatocytes, respectively. The kidney weights were again decreased and there was a slight elevation of serum UN values in males at the

top exposure level. However, in contrast to the results at 6 and 12 months, male mice did not have decreased hepatocytic vacuolation and the liver weights of the female mice exposed to 60 ppm were similar to controls.

Histopathology; Neoplastic: A statistically significant increase in incidence of benign lung tumors (bronchioloalveolar adenomas) was observed in male mice at 60 ppm (22/50 vs. 9/50 in controls). The incidence of bronchioloalveolar adenomas in male mice at 5 or 20 ppm (6/50 and 13/50, respectively) was not affected by exposure. The incidence in males at 60 ppm was higher than the range of historical control values for this tumor type in male B6C3F1 mice (7-32%).

The increased incidence of benign lung tumours in mice is specific to the inhalation route of exposure, since there was no dose related increase in the incidence of lung tumors in B6C3F1 mice following dietary administration of up to 50 mg/kg/day Telone II (Anonymous 57, 1995). At the lower dose the incidence of tumors is greater than at the high dose, and tumors are also present in the concurrent control, and in historical control at a higher incidence.

Table 89: Incidence of lung tumours in Anonymous 57, 1995

Sex	**Male**				**Female**			
Dose (mg/kg/day)	**0**	**2.5**	**25**	**50**	**0**	**2.5**	**25**	**50**
Number examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Adenocarcinoma, bronchioalveolar, malignant, primary, No metastasis	2	4	1	0	0	0	0	0
(Two)	1	0	0	0	0	0	0	0
Adenocarcinoma, bronchioalveolar, malignant, primary, Metastasis	0	0	0	0	0	1	0	0
Adenocarcinoma, bronchioalveolar, malignant, primary, Metastasis or No metastasis, one or two	**3**	**4**	**1**	**0**	**0**	**1**	**0**	**0**
Adenocarcinoma (lacrimal gland) malignant, secondary	0	0	0	0	0	0	1	0
Adenoma, bronchioalveolar, benign, primary	4	9	9	9	3	8	4	5
Adenoma, bronchioalveolar, benign, primary (two)	0	1	0	2	0	0	0	0
Adenoma, bronchioalveolar, benign, primary (three)	1	0	0	0	0	0	0	0
Adenoma, bronchioalveolar, benign, primary (one, two or three)	**5**	**10**	**9**	**11**	**3**	**8**	**4**	**5**
Carcinoma (kidney) malignant secondary	0	0	0	1	0	0	0	1
Carcinoma (liver) malignant secondary	3	0	0	1	0	0	0	0
Total animals with adenoma and/or carcinoma, bronchioalveolar	**6**	**13**	**10**	**11**	**3**	**9**	**4**	**5**

Table 90: Relevant HCD for B6C3F1 Lung Tumours (Anonymous 57, 1995)

Year	**Number of Mice**	**Exposure Route**	**Adenoma**		**Carcinoma**		**Combined adenoma and Carcinoma**	
			Male	**Female**	**Male**	**female**	**Male**	**Female**
1981	96	Dietary	6 (6.3%)	4 (4.2%)	2 (2.1%)	0	ND	ND
1983	86	Dietary	7 (8.1%)	8 (9.3%)	2 (2.3%)	0	7 (8.1%)	8 (9.3%)
1984	50	Dietary	12 (24%)	3 (6%)	2 (4%)	2 (4%)	12 (24%)	3 (6%)
1984	50	Inhalation	10 (20%)	3 (6%)	2 (4%)	1 (2%)	10 (20%)	3 (6%)
1985	50	Dietary	7 (14%)	7 (14%)	2 (4%)	1 (2%)	7 (14%)	7 (14%)
1986	50	Dietary	5 (10%)	3 (6%)	3 (6%)	2 (4%)	5 (10%)	3 (6%)
1990	50	Dietary	12 (24%)	2 (4%)	3 (6%)	1 (2%)	12 (24%)	2 (4%)
1990	50	Dietary	12 (24%)	2 (4%)	2 (4%)	2 (4%)	14 (28%)	4 (8%)
1990	50	Dietary	10 (20%)	2 (4%)	2 (4%)	0	12 (24%)	2 (4%)
1991	50	Dietary	12 (24%)	4 (8%)	1 (2%)	0	12 (24%)	4 (8%)
1992	50	Dietary	12 (24%)	4 (8%)	2 (4%)	1 (2%)	12 (24%)	4 (8%)
1995	50(M)/48 (F)	Dietary	14 (28%)	5 (10.4%)	6 (12%)	2 (4.2%)	14 (28%)	5 (10.4%)
1995	50	Dietary	18 (36%)	ND	1 (2%)	NA	19 (38%)	ND
1995	50	Dietary	9 (18%)	2 (4%)	0	2 (4%)	ND	ND
1997	50	Dietary	15 (30%)	7 (14%)	1 (2%)	0	15 (30%)	7 (14%)
1997	50	Dietary	14 (28%)	4 (8%)	3 (6%)	1 (2%)	14 (28%)	4 (8%)
1998	50	Inhalation	9 (18%)	8 (15%)	5 (10%)	0	9 (18%)	8 (16%)

ND = No Data

Other statistically significant alterations included a lower incidence of non-neoplastic lesions of the kidneys, brain, gallbladder, mediastinal lymph nodes and thymus in one or both sexes of exposed animals relative to controls. Also, a higher incidence of non-neoplastic changes in the larynx and ovaries of exposed mice was

reported. Males at 60 ppm were observed to have fewer total lymphoreticular tumors and fewer total and nonmetastatic liver tumors than control animals, and males at 20 ppm also had significantly fewer non-metastatic liver carcinomas.

Table 91: Tumour incidence. Two years (Summary of most prominent observations) (Anonymous 56, 1987)

Tumour Incidence. 2 years. (Summary for most prominent observations)								
Sex	**Male**				**Female**			
Exposure concentration (ppm)	**0**	**5**	**20**	**60**	**0**	**5**	**20**	**60**
Liver (Nº Tissues examined)	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Adenoma, Hepatocellular, benign, primary	13	14	8	11	8	6	7	9
Adenoma, Hepatocellular, benign, primary(two)	2	3	3	0	1	0	1	0
Adenoma, Hepatocellular, benign, primary (three)	0	0	2	0	0	0	0	0
Carcinoma, hepatocellular, malignant, primary	11	5	3	3	1	1	0	1
Carcinoma, hepatocellular, malignant, primary(two)	0	0	0	0	0	0	1	0
Carcinoma, hepatocellular, malignant, primary(three)	0	1	0	0	0	0	0	0
Carcinoma, hepatocellular, malignant, primary, metastasis	0	1	1	1	0	0	0	0
Lungs (Nº Tissues examined)	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Adenocarcinoma, bronchioloalveolar, malignant, primary, no metastasis	0	0	1	0	0	0	0	0
Adenoma Bronchioloalveolar, benign, primary	9	6	11	20	3	3	4	3
Adenoma Bronchioloalveolar, benign, primary (two)	0	0	2	2	0	0	1	0
Adenoma Bronchioloalveolar, benign, primary (three)	0	0	0	0	1	0	0	0
Adenoma Bronchioloalveolar, benign, primary (Any number)	**9**	**6**	**13**	**22**	**4**	**3**	**5**	**3**
Stomach (Nº Tissues examined)	**50**	**50**	**50**	**50**	**50**	**9**	**4**	**50**
Squamous papilloma, non-glandular mucosa, benign, primary	0	3	2	0	3	2	0	3
Polypoid adenoma, Glandular mucosa, benign, primary	0	0	0	0	0	0	1	0
Urinary Bladder (Nº Tissues examined)	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Carcinoma, malignant, primary, no metastasis	0	0	0	0	0	0	2	0
Papillary Adenoma, benign, primary	0	0	0	0	0	0	1	0
Haemangioma, benign, primary	0	1	0	0	0	0	0	0

The most prominent non-neoplastic treatment-related observation in this study occurred in the transitional epithelium of the urinary bladder and nasal mucosa of mice at 20 ppm and/or 60 ppm. Bladder epithelial effects occurred in both sexes at 20 and 60 ppm, while nasal mucosal effects occurred in both sexes at 60 ppm, and females only at 20 ppm. Hyperplasia of the bladder epithelium has also been reported in other studies, demonstrating the slow progression of this lesion with increasing exposure duration and an apparent sex difference in sensitivity (males were less severely affected than females).

A slow progression in the development of lesions of the olfactory epithelium of the nasal mucosa was also observed. However, in the present study, the lesion was not observed in the majority of mice exposed to 60 ppm Telone II until 24 months of exposure; no lesions of this epithelium were reported after 6 months of exposure, and only a few were affected after 12 months of exposure (Anonymous 55, 1985).

Hyperplastic changes of the respiratory epithelium were reported in both sexes at 60 ppm following exposure for 6 or 12 months (Anonymous 55, 1985). The lesions present at 24 months were of similar severity to those occurring at the previous intervals. It is important to note that even the most severe of these nasal mucosal lesions still involved less than approximately 10% of the respective epithelium present and only those areas of the mucosa having the most contact with inhaled air were affected.

Another treatment-related effect which was considered to be of toxicologic significance was slight hyperplasia of the epithelial lining of the non-glandular portion of the stomach of male mice (only) at 60 ppm. The development of this lesion was attributed to the ingestion of test material which was absorbed in respiratory tract secretions and subsequently swallowed, and during grooming.

The only tumorigenic response, which was observed in the present study, was an increased incidence of benign lung tumors (bronchioloalveolar adenomas) in male mice exposed to 60 ppm vapors (22/50 vs. 9/50 in

controls). No treatment-related tumorigenic response was identified in males exposed to lower concentrations of vapors nor in female mice.

The no-observed-effect level for TELONE II soil fumigant exposure in the present study was 5 ppm for nonneoplastic effects in both male and female mice and 20 ppm for neoplastic effects in male mice. A neoplastic response was not observed in female mice.

Carcinogenicity: dermal

No data

Human information

No data or studies of carcinogenicity in humans are available.

Other relevant information

Inhalation Exposure in Rodents

1,3-D toxicokinetics in humans appears to be similar to that observed in rodents. Inhalation studies with both humans (Anonymous 69, 1992) and animals have shown that 1,3-D vapors are readily absorbed, conjugated with glutathione (GSH) via glutathione S-transferase (GST), and rapidly excreted in the urine as N-acetyl-(S-3-chloroprop-2-enyl)cysteine (3CNAC), a mercapturic acid metabolite. Thus, the major metabolic pathway for 1,3-D leads to its detoxification and excretion. Ingestion studies in animals have demonstrated that the toxicokinetics of oral exposures are similar to those of inhalation exposures. 1,3-D is unlikely to accumulate in the body.

Rat: The major site of absorption of inhaled 1,3-D in the rat is the lung rather than the nasal mucosa (Anonymous 62, 1985). The localized uptake of vapors in rats exposed to 90 or 150 ppm (409 or 682 mg/m^3, respectively) was examined by surgically isolating the upper and lower respiratory tract. The lower respiratory tract absorbed approximately 50% of inhaled 1,3-D vapors, whereas the upper respiratory tract absorbed only 11%– 16% of vapors. Total absorption rates were approximately 73% and 79% at 409 and 682 mg/m^3, respectively.

In the study by Anonymous 63 (2014) male Fischer 344 rats were exposed to 30, 90 300 and 900 ppm of 1,3-D in air for 3 hours. Inhalation pharmacokinetics of technical-grade 1,3-D in rats, showed that the uptake of 1,3-D did not increase proportionately with increasing exposure concentration due to an exposure level-related decrease in the respiratory ventilatory frequency of rats exposed to 90 ppm or greater and the saturation of elimination of 1,3-D by rats exposed to 300 ppm or greater.

Table 92: Summary of plateau values and normalized values (Anonymous 63, 2014)

	ppm	Plateau Values		Normalized values (1,3-D blood concentration/ ppm exposure_	
		Cis	***Trans***	***Cis***	***Trans***
Exposure Period	30	0.085±0.24 at 1 hour	0.12±0.03 at 1 h	3	4
	90	0.20±0.04 at 1 hour	0.26±0.03 at 1 h	2.1	3
	300	0.89±0.20 at 2-3 hours	1.87±0.27 at 2-3 h	3	6.5
	900	Not observed	Not observed	17	23

Following absorption, both isomers of 1,3-D were rapidly eliminated from the bloodstream in a dose-dependent, biphasic manner characterised by a prominent, rapid elimination phase (2-4 min half-life) followed by a slower elimination phase (30-40 min half-life). Consistent with previous reports, exposure to 90 ppm 1,3-D also produced a decrease in renal and hepatic, but not pulmonary, nonprotein sulfhydryl content.

Overall, the data demonstrated that a combination of saturable metabolism and chemically induced changes in respiration control 1,3-D uptake and body burden in rats. However, only decreases in respiration appear to influence vapour uptake at the exposure levels used in subchronic studies (≤150 ppm).

Mouse: 1,3-D significantly increased the incidence of benign bronchioalveolar tumors in the lungs of male mice in the chronic inhalation study at the highest exposure concentration tested, 60 ppm (272 mg/m^3) but, produced no tumors in rats in a chronic inhalation toxicity study up to the highest tested concentration of 60 ppm (272 mg/m3, 53.22 mg/kg bw/day) Anonymous 58, 1987). A 1,3-D inhalation pharmacokinetic study in mice was conducted to provide additional information to aid in interpretation of the observed difference in tumor incidence.

Anonymous 61 (2015), investigated the absorption and systemic bioavailability in B6C3F1 mice following inhalation exposures to 1,3-Dichloropropene. Separate groups of four mice were exposed for approximately 6 hours, using a nose-only inhalation exposure system, to time-weighted average (TWA) chamber concentrations of 2.5, 4.8, 10.5, 19.8, 59.8, or 150.0 ppm (11.4, 21.8, 47.7, 89.9, 271.4, or 680.8 mg/m^3, respectively) 1,3-D vapour.

Functional respiratory data (breathing frequency, f; tidal volume, Tv; minute volume, Mv) were collected from all animals throughout the exposure periods and analyzed to determine the total inhaled volume of test material (area under the Mv curve; AUCMv). Immediately following exposure, mice were sacrificed, and blood samples were collected and processed from all animals for analysis of 1,3-D blood concentrations.

Inhalation of 1,3-D resulted in a concentration-dependent decrease in respiration rate. This sensory irritant-induced depression in respiration rate resulted in a concentration dependent decrease in the total volume of test atmosphere inhaled during the 6-hour exposure period. Based on these data, the 1,3-D vapor concentration needed to reduce the respiration rate by 50% (RD50) was calculated to be 17.9 ppm. Blood samples from all animals in all exposure groups were analyzed to determine the blood concentration of cis- and trans-1,3-D immediately following exposure. Quantifiable levels of trans-1,3-D were present in all blood samples. Quantifiable cis- 1,3-D levels were present in samples collected from mice exposed to 19.8, 59.8 and 150.0 ppm 1,3-D, but were below the lower limit of quantitation in mice exposed to 2.5, 4.8 and 10.5 ppm 1,3-D. The higher blood levels of the trans-1,3-D in all exposure groups is consistent with the faster metabolism of the cis-1,3-D isomer in vivo.

Toxicokinetic analysis showed that blood levels of the individual cis- and trans-1,3-D isomers as well as the summed blood levels of both isomers exhibited nonlinear kinetics in mice exposed at and above the concentration of 59.8 ppm 1,3-D. These results are consistent with the measured depression in respiration rate at higher exposure concentrations, as well as possible saturation of metabolic clearance at the highest exposure level.

Figure 1: Blood concentration (ng/g) of *cis*-, *trans*-, or total 1,3-D in mice following 6-hour exposure (Anonymous 61, 2015)

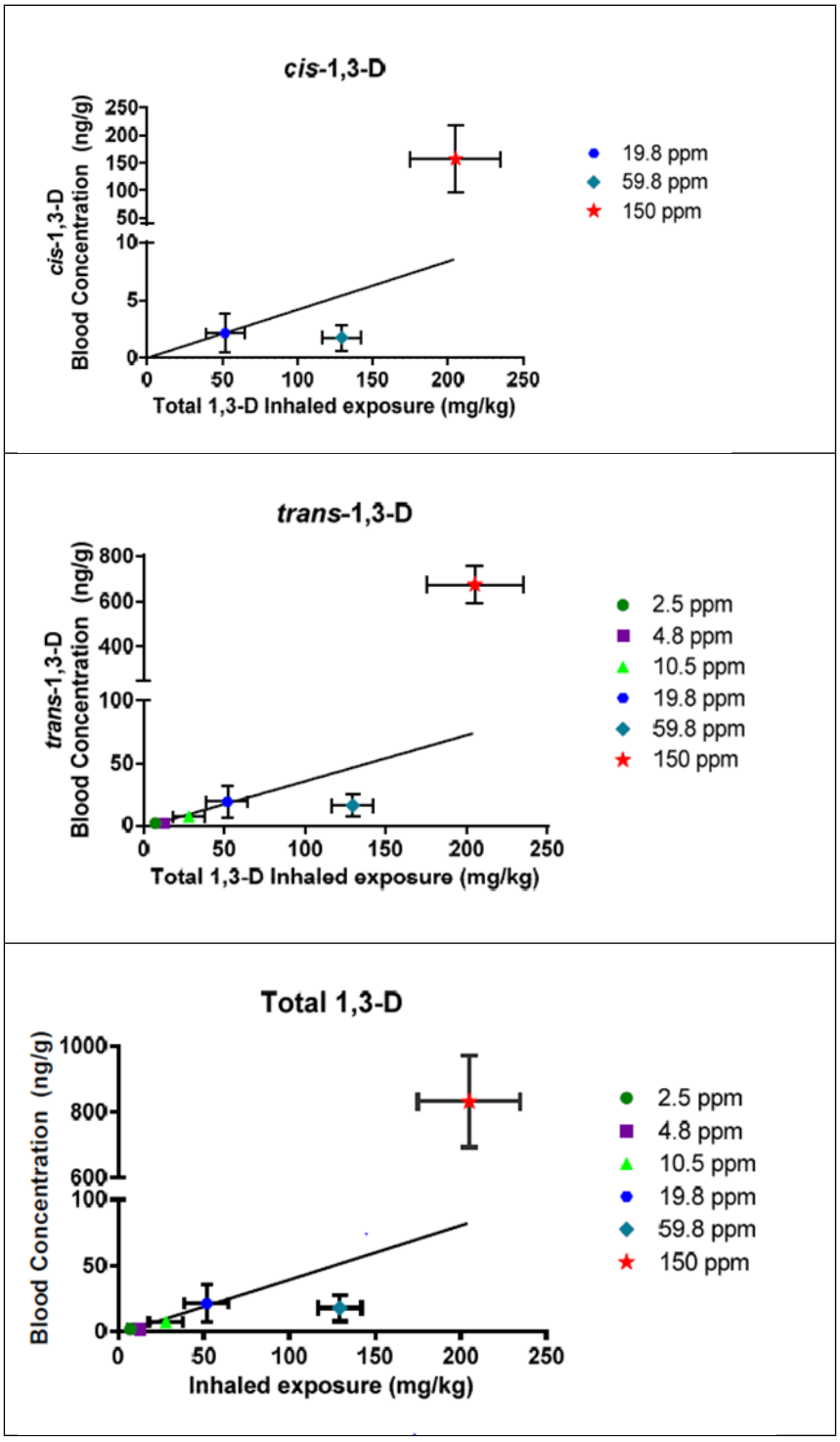


Table 93: Summary of *cis*-, *trans*- and total 1,3-D in blood of mice following inhalation exposure (Anonymous 61, 2015)

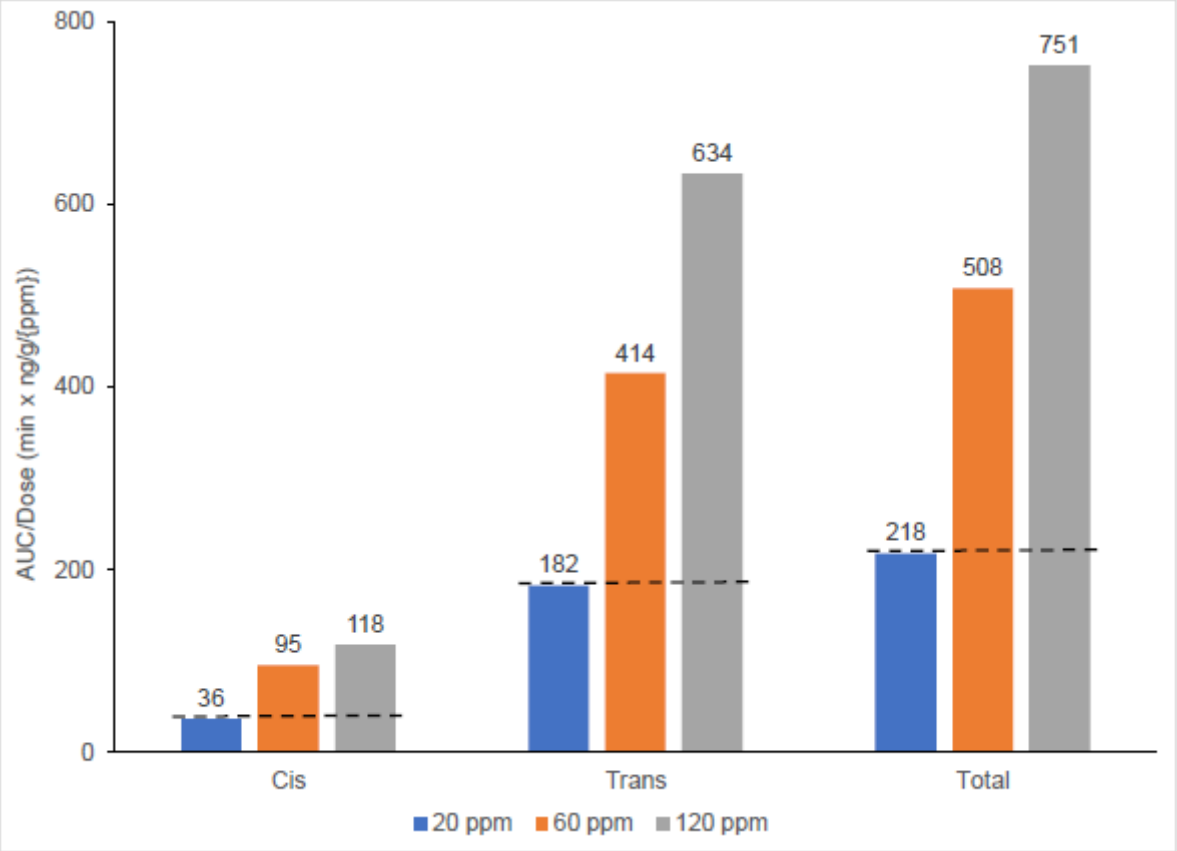
1,3-D Exposure Concentration (ppm)			0	2.5	4.8	10.5	19.8	59.8	150	Dose proportionality
TK Period Exposure in mice (mg/kg)		Mean	0	7.11	12.7	28.0	51.8	129	205	
		SD		1.17	2.59	10.1	12.8	12.8	30.1	
Blood Concentration (ng/g)	*cis* -1,3-D	Mean	[a]0	NQ	NQ	NQ	2.16	1.75	157	sublinear @60 ppm and supralinear @ 150 ppm
		SD					1.68	1.14	61.1	
	trans-1,3-D	Mean	[a]0	1.80	1.77	6.92	19.0	15.9	674	sublinear @60 ppm and supralinear @ 150 ppm
		SD		0.185	0.337	2.32	12.8	9.16	85.5	
	Total 1,3-D (sum of *cis* - and *trans* -)	Mean	[a]0	1.80	1.77	6.92	21.2	17.7	831	sublinear @60 ppm and supralinear @ 150 ppm
		SD		0.185	0.337	2.32	14.4	10.2	138	

NQ: not quantifiable (concentrations were <LLQ)
[a] No Control group was included in this study, default blood level values of zero were used for the TK statistical analysis.

In a more recent study, Anonymous 29 (2018), the steady state pharmacokinetics following repeated nose-only inhalation exposure in male B6C3F1 mice with plethysmography was investigated.

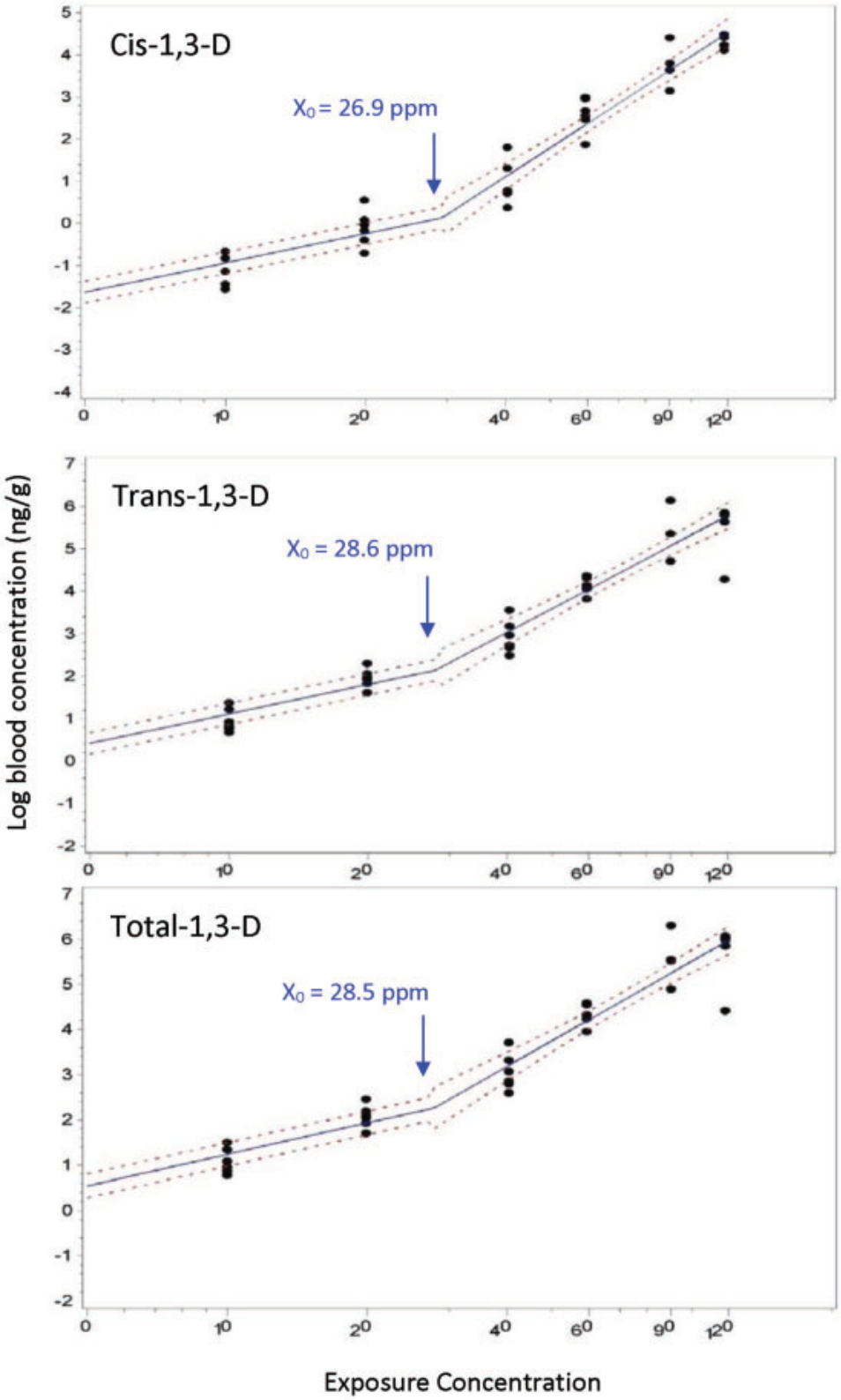
The purpose of this study was to evaluate the steady state blood concentrations of cis and trans isomers of 1,3-dichloropropene (1,3-D) in B6C3F1 male mice following exposure via nose-only inhalation for 15 consecutive days at a duration of up to 6 hours (h) per day. The targeted exposure concentrations were 0, 10, 20, 40, 60, 90 and 120 parts per million (ppm).
In the first experiment of this study time-course data were obtained from mice during and after a 6 hr exposure to 1,3-D. The time-course showed steady-state blood concentrations at 1.5 and 3 hours at 20 and 60 ppm, and by 6 h at 120 ppm. Blood concentrations declined rapidly after exposure (T1/2 = 6 to 13 min). The dose-normalized AUC values at 60 and 120 ppm were higher than the AUC at 20 ppm, indicating saturation of metabolic clearance at and above 60 ppm (Fig. 2). Note that the disproportional increase in AUC385 with respect to exposure concentrations was not due to accumulation following repeated exposures to the test substance since blood concentrations measured prior to exposures on Day 15 were below the limit of detection for animals in the 20, 60 and 120 ppm groups.

Figure 2: Proportionality of Dose-normalized AUC_{385} (Anonymous 29, 2018)



Based on these time-course results, a more comprehensive second experiment was conducted in which steady-state blood levels were measured after 6 hr exposure at 10, 20, 40, 60, 90 and 120 ppm. The results of that second experiment showed that 1,3-D steady state blood levels were linear through 10 and 20 ppm, but became supralinear ~ 30 ppm (Figure 3).

Figure 3: 3-Parameter piecewise linear (hockey-stick) model of 1,3-D toxicokinetics following repeated exposures to B6C3F1 mice (X0 ¼ estimated exposure concentration where the blood concentrations become non-proportional) (Figure from Bartels *et al.*, 2020).



A nonlinear relationship between the concentration of test substance in the exposure atmosphere and the blood concentrations at the end of the 6 h exposure on Day 15 was observed. As exposure increased from 10 to 120 ppm, mean steady-state blood concentrations ranged from 0.360 to 63.3, 2.71 to 283, and 3.08 to 346

ng/g blood for *cis*, *trans* and total (*cis* + *trans*) isomers of 1,3-D, respectively. A 3-parameter "hockey stick" model was used to estimate a statistical point of departure from linear kinetics at concentrations of 30 ppm and above. When modeled non-compartmentally, areas-under-the-curve through 385 minutes (AUC_{385}) were estimated to be 4.3, 30.1, and 88.6 min·µg/g based on estimates of total 1,3-D (both isomers) with respect to time for the 20, 60 and 120 ppm exposure groups.

Table 94: Group mean 1,3-D blood concentrations on day-15 of exposure, and AUC_{385} and dose normalized AUC_{385} for 20, 60 and 120 ppm exposures. (Anonymous 29, 2018)

Exposure conc. (ppm)	Time (min)	Blood Concentration (ng/g)					
		Cis 1,3-D		Trans 1,3-D		total	
		Mean	SD	Mean	SD	Mean	SD
0	360	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
10	360	0.36	0.123	2.71	0.79	3.08	0.9
20	pre	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
	90	3.94	0.82	16.4	3.1	20.3	3.9
	180	1.92	0.62	10.8	1.9	12.7	2.4
	360	0.968	0.433	7.19	1.66	8.16	2.09
	365	0.329	0.142	2.4	0.59	2.73	0.73
	370	0.225	0.035	1.52	0.17	1.75	0.2
	375	0.17	0.1	1.22	0.49	1.39	0.58
	385	0.093	0.031	0.699	0.296	0.79	0.32
	AUC385	**708**		**3631**		**4333**	
	AUC385_D	**35.6**		**182**		**218**	
40	360	2.93	1.72	20	8.5	23	10.2
60	pre	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
	90	20.7	16.5	89.7	69.6	110	86
	180	18	3.3	77.3	7.9	95	10
	360	14.2	5	63.5	12.1	77.7	17
	365	3.43	3.91	25.7	30.6	29.2	34.5
	370	1.74	1.26	12.5	11.7	14.2	12.9
	375	0.544	0.163	3.63	0.69	4.17	0.83
	385	0.24	0.082	1.86	0.78	2.1	0.86
	AUC385	**5638**		**24610**		**30171**	
	AUC385_D	**94.9**		**414**		**508**	
90	360	47.1	25	251	151	298	176
120	pre	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
	90	20.8	15.4	146	67	167	82
	180	43.6	19.3	244	74	287	92
	360	63.3	27.8	283	106	346	132
	365	26.2	9.9	203	73	229	83
	370	12.1	7	115	76	128	82
	385	3.96	0.22	53.7	12.1	57.6	12.1
	400	1.98	1.09	21.7	8.8	23.7	9.8
	AUC385	**13895**		**74825**		**88637**	
	AUC385_D	**118**		**634**		**751**	

BLOQ – below the limit of quantitation; Pre – pre-exposure bleed; SD – standard deviation; AUC_{385} = min*ng/g; AUC385_D = min*ng/g/{ppm}

Table 95: Summary of Hockey-Stick 3-Parameter Models (initial slope b1 = 1) used for Statistical Dose Proportionality Analysis. (Anonymous 29, 2018)

Analyte	Method	Estimate, X_0 (ppm)	LCB	UCB
Cis-1,3-D	1	26.93	16.80	37.06
	2	29.32	22.27	36.37
	3	28.55	18.06	39.05
	4	30.84	23.50	38.18
Trans-1,3-D	1	28.61	18.94	38.29
	2	30.83	24.13	37.54
Total-1,3-D	1	28.46	18.77	38.15
	2	30.72	24.05	37.38

Method 1: All data, cis-1,3-D concentrations >LOD but <LOQ not censored
Method 2: Without animal 613, cis-1,3-D concentrations >LOD but <LOQ not censored Method 3: All data, censoring for cis-1,3-D modeled
Method 4: Without animal 613, censoring for cis-1,3-D modeled
X_0, estimated concentration (ppm) of 1,3-D in air that causes nonlinear kinetics
LCB, UCB, Lower and Upper Confidence Bounds of the 95% confidence interval for the estimate of X_0; units are ppm 1,3-D in air

These steady-state and time-course data taken together are consistent with saturation of physiological clearance mechanisms for animals exposed to atmospheres containing greater than 30 ppm of the test substance and, therefore, 30 ppm should be considered as a kinetically derived maximum dose (KMD) for repeated exposures of 1,3-D in the mouse. Concentrations above this KMD would not be considered relevant to human hazard assessment.

Effects on respiration were also measured using head out plethysmography for animals in the 20, 60 and 120 ppm exposure group. Acute and delayed effects were observed in the plethysmography. On Day 1, a dose-dependent decrease in the mean respiratory rate from 167.9 to 123.5 breaths/min was observed as exposure concentrations increased from 20 to 120 ppm. Tidal volumes were consistent between groups, so a similar dose-dependent decrease was also observed in mean minute volumes which ranged from 29.6 to 23.0 mL/min for the 20 ppm and 120 ppm groups, respectively. On Day 14, group mean respiratory rates further decreased ranging from 164.8 to 82.3 breaths/min for animals in the 20 and 120 ppm exposure groups, respectively. The 33% reduction in respiratory rate from Day 1 in the 120 ppm exposure group was offset by a 22% increase in tidal volume such that net decreases in the mean minute volume were comparable across groups. When group mean minute volumes were used to estimate inhaled doses, a linear relationship was observed for the 20 and 60 ppm exposure groups, but a lower than expected dose was observed for the 120 ppm group. This suggests the disproportion observed at 120 ppm is underestimated considering the nominal increase from 60 ppm to 120 ppm does not correlate to a doubling of inhaled dose.

The reaction of 1,3-D with glutathione (GSH)

The reaction of 1,3-D with glutathione (GSH) was investigated by incubation of a mixture of the cis- and trans-isomers of 1,3-D with GSH in the presence and absence of fractions of rat liver (Anonymous 68, 1977). These experiments show that cis-1, 3-D is metabolised by an enzyme in rat liver cytosol (likely the glutathione S-alkyl transferase) which requires GSH for activity. The spontaneous reaction of cis-1,3-D with glutathione is slow, however an enzyme-catalysed reaction in the presence of GSH is rapid and is the most important mechanism for the destruction of the 1,3-D in the system used. The trans-isomer (in the presence of cis-isomer) was degraded 4-5 times slower than the cis-isomer in the presence of the enzyme and GSH.

A mechanistic study using GSH levels as an endpoint showed that 1,3-D at doses used in chronic bioassays depleted non-protein sulfhydryl (NPS) content (routinely used as an indicator of tissue GSH content) in glandular and non-glandular stomach, liver and kidneys of Fischer 344 male rats (Anonymous 60, 1982). Blood concentration time-profile of 1,3-D following oral administration was also investigated. Oral administration

of 1,3-D (50 mg/kg bw) produced significant alterations in non-protein sulfhydryl content in the liver, kidney, forestomach and glandular stomach of male rats. Data collected are described in the table below.

Table 96: Tissue NPSH levels (% of control values, means) (Anonymous 60, 1982)

Post-Dosing Time (hr)	Liver	Kidney	Bladder	Forestomach	Glandular Stomach
2	63.4*	89.4*	94.7	14.3*	62.3*
4	54.0*	101.9	96.6	20.0*	63.3*
8	81.3*	108.2	103.6	83.2*	94.4
12	102.8	102.9	82.2	161.6*	113.0*
24	108.0	98.5	92.2	211.7*	114.6

*Statistically different from control by Student's t test ($p \leq 0.05$).

Blood concentrations of unchanged 1,3-D were not detectable at 1.5, 4, 8- and 12-hours post-dosing. At 0.25 hour cis- and trans-isomer were determined to be 6.58 ppb and 11.72 ppb, respectively. The trans isomer was also detected at 0.75 hour, representing 11.72 ppb. The trans-isomer was detected in blood for a longer period than cis-1, 3-D because of a higher reactivity of cis-isomer than trans-isomer.

Table 97: Blood concentrations of *cis*- and *trans*-1, 3-D (Anonymous 60, 1982)

Post-Dosing Time (hr)	Blood 1,3-D Concentration (ppb)	
Cis	Trans	
0.25	6.58	11.72
0.75	ND	8.38
1.5	ND	ND
4	ND	ND
8	ND	ND
12	ND	ND

ND= blood concentrations of unchanged 1,3-D were not detectable at these time periods. N=3 rats/time period at 4-, 8- and 12-hours post-dosing and N=1 rat/time at 0.25, 0.75- and 1.5-hours post-dosing.

Oral administration of 50 mg/kg 1,3-D produced significant depression of non-protein sulfhydryl content in glandular and non-glandular stomach, liver and kidneys.

The most depressed values were found in non-glandular stomach. The marked rebound observed in forestomach non-protein sulfhydryl content at 12- and 24-hours post-dosing is a characteristic commonly seen in tissues that have undergone severe non-protein sulfhydryl depletion.

Detectable blood concentrations of unchanged 1,3-D were only found at time periods immediately after dosing. This is associated with a first-pass effect via conjugation of most, if not all, of the administered dose of 1,3-D with GSH in the stomach and liver prior to reaching the systemic blood. The trans-isomer was detected in blood for a longer period than cis-1, 3-D because of a higher reactivity of cis-isomer than trans-isomer.

GSH conjugation is an important pathway for the depression of forestomach, glandular stomach, liver and kidney non-protein sulfhydryl content observed in this study suggesting that the ability of the rat to detoxify 1,3-D in this study may be compromised at an oral dosage level of 50 mg/kg.

In another study looking at the pharmacokinetics of 1,3-D and effect of 1,3-D on tissue non-protein sulfhydryls, and macromolecular binding in Fischer 344 Rats and B6C3F1 mice following oral administration (Anonymous 59, 1985), Fischer 344 rats were administered single oral dose of 1,3-dichloropropene, at two dose levels: 1 and 50 mg/kg. B6C3F1 mice were also administered single oral dose of 1,3-dichloropropene, at two dose levels: 1 and 100 mg/kg. Over the range of administered dose levels, 1,3-D was rapidly metabolised with approximately 69-76% (rats) and 76-93% (mice) of the administered doses radioactivity recovered as either urinary metabolites and CO_2 within 48 hours post-dosing. The predominant routes of ^{14}C excretion in rats and mice following a single oral dose of ^{14}C-*cis/trans*-1,3-D were via the urine, faeces and expiration of $^{14}CO2$. Urinary excretion was the major route of ^{14}C elimination in rats and mice following a single oral dose of ^{14}C-1, 3-D. Half-lives of urinary excretion were 5-6 hrs. Analysis of rat and mouse urine revealed no unchanged parent compound and two major 1,3-D metabolites. Since these metabolites were identified in rat urine as the mercapturic acid of 1,3-D and its corresponding sulfoxide (or sulfone), conjugation with glutathione appears to play an important role in the metabolism of 1,3-D by rodents. In rats, at high dose levels, at 50 mg/kg,

metabolite profiles suggested that oxidation route from mercapturic acid to its sulfoxide was saturated. Comparing urinary profiles between *cis/trans*-1, 3-D mixture and only *cis*-1, 3-D, results suggested more reactivity of cis-isomer with this metabolite pathway than trans-isomer. Levels of radioactivity in faeces appear to reflect the presence of ingesta in the stomachs of dosed rats and mice and its potential covalent binding of ^{14}C-1, 3-D. The excretion and tissue distribution of ^{14}C in rats and mice were independent of administered dose, suggesting that over the range of administered dose levels the disposition of 1,3-D follows 'linear' pharmacokinetics. Levels of radioactivity after 48 hours were detected in almost all tissues analysed. Depletion and macromolecular binding were noted in several tissues of both species following the oral administration of 1.3-D. These effects were more pronounced in the non-glandular stomach suggesting that the non-glandular stomach of rats and mice may be sensitive to the potentially toxic effects of 1, 3-D following repeated oral administration, particularly at dose levels of 25 to 100 mg/kg, where NPS depletion and/or macromolecular binding are relatively pronounced. However, the reason why other organs in which statistically significant NPS depletion was also observed at the same doses (glandular stomach and liver) are not so sensitive to potential toxic effects of 1,3-D, remains to be explained.

In the study by Anonymous 66 (1996), the glutathione transferase activities of several mammalian cell lines were evaluated. In this study Chinese hamster ovary cells (CHO-K1-BH4) and Chinese hamster lung cell lines DEDE (CCL39) and DON (CCL16) were cultured *in vitro*. In addition, rat hepatocytes were isolated from male Fischer 344 rats and cultured. The net activities of the mixed isozymes of GST recovered from harvested cells and male Fischer 344 rat liver were determined for the alkyl-substrate 1,3-D, the aryl-substrate CDNB, and the aralkyl-substrates NPEB and TPBO. In addition, the net activities of the mixed isozymes of GST recovered from B6C3F1 mice for 1,3-D substrate were measured *in vitro*.

GST activities observed with the 1,3-D substrate were much lower than those observed with CDNB and displayed pronounced differences dependent upon the source of the enzymes. Interestingly, the activity of GST increased with increasing levels of sophistication and proximity to the whole animal.

Table 98: Glutathione transferase activity of 100,000 g supernatant (cytosol) recovered from cells employed in genotoxicity assays. 1,3-D substrate (Anonymous 66, 1996)

Source	1,3-D (nmoles product/min/mg protein). 4-6 assays
Mouse liver cytosol	83.8 ± 9.5
Rat liver cytosol	119 (mean of 7 replicates using tissues from a single rat)
Primary rat hepatocytes	21.1 ± 3.9
CHO	3.22 ± 2.25
CHL (DEDE)	13.4 ± 3.5
CHL (DON)	9.43 ± 2.98
Salmonella typhimurium	<0.1 (data using cis-1, 3-D only)

Activities observed with the other two substrates, indicative of aralkyl-substrate conjugation activities, were generally even lower, with the exceptions of the NPEB substrate with CHO and CHL (DON cell line) cytosols which were similar to that observed with 1,3-D. 1,3-D conjugation activity was greatest with more complex sources of GSTs such as found in vivo. These results are consistent with the hypothesis that genotoxic potential of 1,3-D may be mitigated in the presence of physiological levels of GSH and GST activity.

Anonymous 65 (1997) Investigated the effects of 1,3-D on DNA synthesis (cell proliferation) and apoptosis in target tissues in rats (liver) and mice (lung and urinary bladder). In addition, a sensitive assay of DNA adduct formation *in vivo*, the 32P-Post Labelling Assay, was conducted to further characterise any genotoxic potential, direct or indirect, of 1,3-D in selected tissues. No clear-cut evidence of an effect on either cell proliferation or apoptosis rates in target tissues were observed. The 32P-Post Labelling Assay revealed neither any unique DNA adducts, nor any statistical elevation in the levels of normally occurring adducts in either rat liver or mouse lung DNA. There were no treatment-related histopathologic effects in the livers of male rats treated during 25 days or in the lungs or urinary bladder of male mice exposed for 26 days. However, the urinary bladder was the target organ in females, and these mechanistic studies were not realized in female mice. The mechanistic studies presented to explain the tumours were not conclusive. Only a dose-related decrease in tissue (liver rats and lung mice) GSH levels of treated animals was observed.

Anonymous 64 (1998), went on to histopathologically evaluate the rat liver, mouse lungs and mouse bladders from the animals in the previous study. There were no treatment-related histopathologic effects in the livers of male Fischer 344 rats administered 1,3-D in corn oil vehicle, given by oral gavage 5 days/ week, for 3, 12 or 25 days at dose levels of 0, 5, 12.5, 25 or 100 mg/kg bw/day.

There were no treatment-related histopathologic effects in the lungs or urinary bladder of male B6C3D1 mice exposed to 0, 10, 30, 60 or 150 ppm 1.3-D vapours, 6 hours/day, 5 days/week, for 3, 12 or 26 days. However, the urinary bladder was a target organ in females, and these mechanistic studies were not conducted in female mice.

The purpose of these mechanistic studies was to evaluate the effects of 1,3-D on DNA synthesis (cell proliferation) and apoptosis in target tissues in rats (liver) and mice (lung and urinary bladder). The mechanistic studies presented to explain the tumours were not conclusive. Only, a dose-related decrease in tissue (liver rats and lung mice) GSH levels of treated animals was observed.

In 2003, Anonymous 30, investigated the possible mechanism of 1,3-D induction and/or promotion of lung tumours in male A/J mice. The study involved two phases: tumour initiation with intraperitoneal vinyl carbamate (VC) known to induce lung tumours in A/J mice, and the potential tumour promotion or enhancement of tumours with 1,3-D by inhalation (0 or 60 ppm).

Tumour initiation: vinyl carbamate (16 mg/Kg bw) in pyrogen-free buffered saline was injected interperitoneally twice (14 days and 7 days prior to the start of inhalation exposures).

Tumour Promotion: 1,3-D (Lot.V1253, purity 99.2%) inhalation exposure (60 ppm administered six hours per day, five days per week for approximately 25 weeks) beginning when the mice were approximately seven weeks of age.

Strain A/J mice exhibit spontaneous lung tumours as they age and are very sensitive to the effects of many carcinogens. In the present study, 1,3-D caused an increase in adenoma incidence and adenoma volume, which correlated with observed elevation in cellular proliferation within the quantifiable adenomas. While not affecting proliferation rates in normal lung tissue, the selective enhancement of tumour cell proliferation by 1,3- D indicates the compound has properties associated with other tumour-promoting agents. A lung tumour incidence 10%, 26% and 100% was seen in control, 1,3-D treated, and VC treaded animals respectively. Additionally, in the current study it appears that 1,3-D was promoting the growth of pre-existing spontaneously formed lesions. Thus, inhaled 1,3-D bioactivity in this study can be interpreted as weakly genotoxic or a non-genotoxic promoter of spontaneous lung adenomas. The effects of 1,3-D on the growth of VC-induced lesions was not apparent from this study, however this is likely due to the large tumour burden caused by VC treatment.

Table 99: Summary of lung adenoma incidence and volume of lung adenomas in male mice, and BrdU labelling index of normal lung and lung adenomas (Anonymous 30, 2003)

Incidence and number of lung adenomas in male mice.				
Group	**Treatment**	**Lung Tumour Incidence**	**Total tumours in animals at risk**	**Mean tumours in animals at risk**
1	Control	2/20(10%)	2/20	0.10 ±0.31
2	1,3-D	5/19(26%)	6/19	0.32 ±0.58
3	VC	20/20(100%)	976/20	48.80 ±9.40[a]
4	VC+1,3-D	20/20(100%)	980/20	49.00 ±9.21 [a]

a indicates statistical significance from control group (group 1)

Number and volume of lung adenomas in male mice				
Group	**Treatment**	**Total tumours in animals with tumours**	**Mean of tumours in animals with tumours**	**Relative adenoma volume**
1	Control	2/2	1.00 ± 0.00	0.04 ± 0.06
2	1,3-D	6/5	1.20 ± 0.45	0.17 ± 0.33
3	VC	976/20	48.80 ± 9.40 [a]	7.23 ± 2.62 [a]
4	VC+1,3-D	980/20	49.00 ± 9.21 [a]	7.17 ± 3.77 [a]

a indicates statistical significance from control group (group 1)

The BrdU labelling index in normal lung and lung adenomas in male mice			
Group	**Treatment**	**Non-adenoma Tissue**	**Adenoma Tissue**

		Labelling Index %		Labelling Index %
		Type I	Type II	
1	Control	9.63 ± 1.54	12.53 ± 5.68	16.47 ± 4.03
2	1,3-D	11.06 ± 2.42	11.82 ± 5.68	20.44 ± 5.99
3	VC	11.32 ± 3.72	15.20 ± 3.94	13.85 ± 3.09 a
4	VC+1,3-D	11.97 ± 4.98	16.78 ± 7.34	14.02 ± 2.63 [a]

a indicates statistical significance from control group (group 1)

In 2014, Anonymous 63, assessed potential nuclear receptor activation of 1,3-dichloropropene using rat primary hepatocytes through the assessment of biomarker gene expression responses. Gene expression responses were determined by quantitative real-time PCR for Cyp1a1, Cyp2b1, Cyp3a1 and Cyp4a1 as biomarkers for activation of AhR CAR, PXR and PPAR-α signalling pathways, respectively.

Replicate 1

Positive control compounds demonstrated an acceptable assay response with a minimum induction of at least 10-fold for each CYP biomarker gene.

Table 100: Prototypical inducers for CYP Genes – Positive control compounds

Prototypical inducers for CYP Genes – Positive control compounds		
Chemical name	**Final Dosing Concentration**	**Receptor**
3-Methylcholanthrene	10 µM	AhR
Phenobarbital	500 µM	CAR
Dexamethasone	50 µM	PXR
WY 14643	10 µM	PPARα

AhR-aryl hydrocarbon receptor, CAR – constitutive androstane receptor, PXR – pregnane X receptor, and PPARα – peroxisome proliferator-activated receptor alpha

Six targeted dose levels of 3, 10, 30, 100, 300, and 1000 µM of 1,3-dichloropropene were used in the replicate 1 assay. Treatment with 1,3-dichloropropene did not result in CYP biomarker gene induction responses that were ≥ 40% of the positive control response at any of the evaluated concentrations. 1,3-dichloropropene considerably increased cytotoxicity (cell viability < 70%) at the concentrations of 300 µM and higher (300 & 1000 µM). Based on these results, 1,3-dichloropropene was considered to be negative for nuclear receptor activation in rat primary hepatocytes in replicate 1.

Replicate 2

Positive control compounds of nuclear receptor PXR and PPAR-α demonstrated an acceptable assay response with a minimum induction of at least 10-fold. In this study, the induction of Cyp1a1 and Cyp2b1 did not meet the 10-fold minimum induction; however, the specificity of the response for the respective nuclear receptor and the elevated baseline levels of transcript demonstrated the robust nature of the induced response. Therefore, this run (#2) was considered acceptable. Compared to the vehicle control (DMSO), Cyp1a1, Cyp2b1, Cyp3a1, and Cyp4a1 were induced 7.8-, 8.3-, 12.1-, and 200.5-fold by their respective positive control compounds (3-MC, PB, DEX, and WY).

As in replicate 1, 1,3-dichloropropene increased cytotoxicity (cell viability < 70%) at concentrations of 300 µM and higher. Therefore, dose concentrations in replicate 2 were redefined to further characterize the dose response and to obtain more meaningful data. Therefore, in replicate 2, 1000 µM concentration was replaced with 1 µM concentration.

Treatment with 1,3-dichloropropene did not result in CYP biomarker gene induction responses that were ≥ 40% of the positive control response at any of the evaluated concentrations. Cytotoxicity (cell viability < 70%) was observed at concentrations of 100 & 300 µM. Based on these results, 1,3-dichloropropene was considered to be negative for nuclear receptor activation in rat primary hepatocytes in replicate 2.

Under the conditions of this study, 1,3-D did not activate AhR, CAR, PXR or PPARα nuclear receptor in rat primary hepatocytes.

Summary and discussion of carcinogenicity

Studies of 1,3-dichloropropene in rodents (rats and mice) using the inhalation and oral (gavage and diet) routes showed chronic toxicity and neoplastic changes dependent on the species and route of administration used.

Rats:

Chronic oral gavage administration of DD-92 in Sprague Dawley rats at 2, 10 or 25 mg/kg bw/day was associated with increased incidence and/or severity of hyperplasia and hyperkeratosis of the stratified squamous epithelium lining the fore stomach in males and females at 10 mg/kg/day. DD-92 did not result in any oncogenic effects. However, the high mortality rate on this study is considered to have compromised its reliability.

Chronic dietary administration of Telone II (microencapsulated) in Fischer 344 rats dosages of 0, 2.5, 12.5 and 25 mg/kg bw/day was associated with treatment-related observations in the stomachs (basal cell hyperplasia of the non-glandular mucosa of the stomach in males and females at 12.5 or 25 mg/kg bw/day, which displayed no evidence of progression from the 12-month time point to the 24-month time point. The incidence of benign liver tumours (hepatocellular adenomas) was statistically increased in males and females by a trend test at 25 mg/kg bw/day. Pairwise comparison of tumour incidence, however, indicated a statistically significant increase only in male rats (males: 9/50 at 25 mg/kg, 6/50 at 12.5 mg/kg vs 2/50 in controls; females 4/50 at 25 mg/kg vs 0 in controls). Historical background incidence for hepatocellular adenoma in six studies with F344 rats from the same laboratory show for female rats, the incidence for hepatocellular adenomas varied from 0/50 to 3/50. For male rats the historical background incidence varied from 0/50 to 2/50. Therefore, the increased incidence of hepatocellular adenomas in males given 12.5 or 25 mg/kg/day and females given 25 mg/kg/ day are outside the historical control range. However, body weights were decreased as much as 15-16% (i.e., body weight of high dose male and females were statistically decreased relative to controls; males were 9-16% lower than controls from week 13 to termination while female body weights averaged 10-15% lower from week 73 to termination) and body weight gains were suppressed an average of 17-19% (i,e., body weight gain for males was 19-26% lower from week 13 to termination with an average decrement of 19% while for females, weight gain decrements were from 10-26% over the entire dosing period with an average decrement of 17%) in rats ingesting 25 mg/kg/day Telone II, indicating that slight increases in the incidence of hepatocellular adenomas were only evident in rats at a dose level that exceeded the maximum tolerated dose. The study reported that the increased incidence in mid-dose males (6/50) was not statistically significant, but did indicate a possible effect at this dose level; however, pairwise comparison of tumor incidence only indicated statistically significant increases in tumor incidence for high-dose male rats. The description and interpretation of the significance of hepatocellular adenomas above reflects knowledge of the HCD available at the time of the study conduct and additional HCD is now available and described in the following paragraph.

Historical control data for hepatocellular adenomas The HCD from 17 carcinogenicity studies ranging from 1991 to 2012 conducted on F344 rats (dietary administration) were collected from the Dow toxicology testing laboratory.

The Dow HCD for hepatocellular adenomas were 0–16% and 0–8%, in male and female F344 rats, respectively, from 1991 to 2012. Importantly, the 16% incidence for males in 1992 and 8% for females in 1997 bracket the time frame of the study of interest (year of 1995). As a result, the incidences of hepatocellular adenomas in F344 rats in mid-dose males (12%) and high-dose females (8%) were within the historical control range (12% vs. 0–16% in males and 8% vs. 0–8% in females); in addition, the incidence of high-dose males is just outside the HCD (18% vs. 16%). From a statistical perspective, no significant increase in tumor (adenoma, carcinoma, or combined) incidence was observed by pairwise comparisons to controls for mid-dose males and high-dose females, although a significant increasing trend was observed for both males and females ($p< 0.05$). Although a $p< 0.05$ was identified for the high-dose male group, the p value of 0.0256 is greater than 0.01. According to Haseman's evaluation of NTP carcinogenicity studies (1983), the overall false positive rate associated with the default NTP decision-making process is about 7%, and could be closely approximated by a statistical decision rule which is in the case of rare tumors, $p< 0.05$, and common tumors, $p< 0.01$ are required. More recent publications have reported similar results (Haseman 1998; Lin and Rahman 1998; Rahman and Lin 2008) and application of Haseman's rule has also been recommended by the FDA (2001).

In summary, the HCD from the Dow toxicology laboratory demonstrates variable background incidences for hepatocellular adenomas in both male and female F344 rats. The liver tumor incidences in mid-dose males and high-dose females are within the relevant historical control ranges. In addition, the lack of statistical significance supports the observation that the incidences of benign adenomas in the mid-dose male and high-dose females were similar to those of controls. In terms of the incidence of hepatocellular adenomas in high dose males being just outside of the HCD range, employing Haseman's rule clarifies the lack of biological significance

Following inhalation exposure (6- and 12-months) to Telone II in male and female Fischer 344 rats at 0, 5, 20 or 60 ppm (0, 22.7, 90.8 and 272 mg/m3, respectively; equivalent to achieved doses of 0, 4.43, 17.74 or 53.22 mg/kg bw/day respectively) there were no treatment-related observable pathologic changes or histopathological changes noted in either sex of interim sacrifice groups of rats. However, it was noted that lesions of the nasal mucosa which had previously been reported to occur at a low incidence in male rats exposed to 30 ppm 1,3-D, and all rats exposed to 90 ppm 1,3-D, for 13-weeks (Anonymous 73, 1984) were not observed in the present study in rats exposed to up to 60 ppm Telone II for up to 12 months, suggesting that this lesion is not progressive in rats with increasing time of exposure and may even resolve with time.

Following 2-years inhalation exposure to Telone II vapours, histopathologic examinations showed a statistically significant increase in incidence of non-neoplastic effects in nasal tissues of male and female rats at 60 ppm. These were unilateral or bilateral decreased thickness of olfactory epithelium, unilateral or bilateral erosions of olfactory epithelium, and unilateral or bilateral submucosal fibrosis (underlying olfactory mucosa). There were no statistically identified increases in any tumour incidence in rats exposed to Telone II.

Mice:

Chronic oral gavage administration of DD-92 in CD-1 mice at dose levels of 2, 10, and 25 mg/kg/day was associated with a spectrum of lesions attributable to chronic irritation of the urinary bladder of females at 25 mg/kg bw/day; transitional cell hyperplasia, and hyaline change of the lamina propria (considered to reflect responses to chronic irritation), stromal hyperplasia, stromal hypertrophy and accumulation of brown pigment in reticuloendothelial cells. There was a slightly increased incidence of benign submucosal mesenchymal tumours in the urinary bladder of female mice at 25 mg/kg bw/day (3/65) compared to controls (0/65). However, the mesenchymal tumours seen in three high-dose females were considered to be a secondary response to chronic irritation and represent a benign proliferation lesion indicating minimal or equivocal neoplastic activity.

Chronic dietary administration of Telone II (microencapsulated) in B6C3F1 mice at doses of 2.5, 25 and 50 mg/kg bw/day was associated with depression of in-life body weight /body weight gain and food consumption at 25 and 50 mg/kg, but no increased incidence of tumours.

Inhalation exposure (6 and 12 months) to Telone II in B6C3F1 mice at 5, 20 and 60 ppm (corresponding to 0, 22.7, 90.8 or 272.4 mg/m3, respectively or equivalent to 0, 7.69, 30.75 and 92.24 mg/kg bw/day, respectively), was associated with histological changes in the urinary bladder in 1/10 male and 4/10 female mice at 60 ppm at 6 months, (moderate hyperplasia of the transitional epithelium, occasionally accompanied by an inflammatory reaction in the lamina propria of the urinary bladder) and focal hyperplasia and hypertrophy of the respiratory epithelium in the nasal turbinates of male and female mice at 60 ppm and males only at 20 ppm, following 6 months. Following 12 months, hyperplasia of the transitional epithelium of the urinary bladder was observed in the majority of females at 60 ppm and in 1/10 females at 20 ppm (simple thickening of the epithelium and involved a variable amount of the mucosa), focal papillary hyperplasia, epithelial pigmentation, and vacuolization were also occasionally present, and the lamina propria underlying the hyperplastic epithelium frequently contained inflammatory cells and prominent fibroblasts, connective tissue and vasculature. At 20 and 60 ppm, similar to the 6-month exposure observations, hyperplasia and hypertrophy of the respiratory epithelium of the nasal turbinates was observed in male and female mice at 60 ppm and males at 20 ppm.

Following inhalation exposure for 2-years to Telone II in B6C3F1 mice, non-neoplastic treatment-related effects occurred in the transitional epithelium of the urinary bladder and nasal mucosa of mice at 20 ppm and/or 60 ppm, and slight hyperplasia of the epithelial lining of the non-glandular portion of the stomach of

male mice at 60 ppm. The development of this lesion was attributed to the ingestion of test material which was absorbed in respiratory tract secretions and subsequently swallowed, and during grooming. The only tumorigenic response was an increased incidence of benign lung tumours (bronchioloalveolar adenomas) in male mice at 60 ppm (22/50 vs. 9/50 in controls). No treatment-related tumorigenic response was identified in males exposed to lower concentrations nor in female mice.

A pharmacokinetic study in male B6C3F1 mice (Anonymous 29, 2018) showed there to be a disproportional increase in AUC_{385} with respect to exposure concentrations. This was not due to accumulation following repeated exposures to the test substance since blood concentrations measured prior to exposures on Day 15 (last day of study) were below the limit of detection for animals in the 20, 60 and 120 ppm groups. These steady-state and time-course data taken together are consistent with saturation of physiological clearance mechanisms for animals exposed to atmospheres containing greater than 30 ppm of the test substance and, therefore, 30 ppm should be considered as a kinetically derived maximum dose (KMD) for repeated exposures of 1,3-D in the mouse. Concentrations above this KMD would not be considered relevant to human risk assessment which considers both hazard and exposure. This statement is predicated on the fact that dose-normalized AUC values show that exposure to 1,3-D in air at 60 ppm and higher afford systemic exposures that are 3-4 times above dose proportionality which is consistent with saturation of metabolic clearance between 20 and 60 ppm. Above 60 ppm probable saturation of GHS-based metabolic clearance leads to the supralinear increase in systemic exposure which is not appropriate for use in human health risk assessment. Therefore, an increase in lung adenomas in male mice was only observed at a dose that exceeded the kinetically derived maximum dose level for repeated exposure via inhalation.

The incidence of lung adenomas in males at 60 ppm was higher than the range of historical control values for this tumour type in male B6C3F1 mice (6.3-36%). However, historical control data show lung adenomas and adenocarcinomas in B6C3F1 mice to be commonly observed tumours, with a fairly high spontaneous incidence in both males and females; however, in almost every study there is a higher incidence in males than females with the highest recorded incidence of 18/50 (36%), in a dietary exposure study. These data indicate that inhalation exposure to 1,3-D at 60 ppm can cause a slight increase in the incidence of what is a fairly common benign tumour type for these animals, it did not reduce the latency period, did not adversely affect survival of the animals, and there was no evidence of progression to malignancy.

In conclusion:

- In mice, 30 ppm should be considered as a kinetically derived maximum dose (KMD) for repeated exposures of 1,3-D. Concentrations above this KMD would not be considered relevant to human hazard assessment because as described previously, there is evidence of non-linear (i.e., supralinear) kinetics at dose levels above this exposure level due to saturation of physiological clearance mechanisms which would lead to systemic exposures well beyond the range for dose proportionality.
- Inhalation pharmacokinetics of 1,3-D in rats, showed that the uptake of 1,3-D did not increase proportionately with increasing exposure concentration due to an exposure level-related decrease in the respiratory ventilatory frequency of rats exposed to 90 ppm or greater and the saturation of elimination of 1,3-D by rats exposed to 300 ppm or greater.
- Analysis of rat and mouse urine revealed no unchanged parent compound and two major 1,3-D metabolites, mercapturic acid of 1,3-D and its corresponding sulfoxide (or sulfone), conjugation with glutathione appears to play an important role in the metabolism of 1,3-D by rodents.
- In rats, at high oral dose levels of 50 mg/kg, metabolite profiles suggested that oxidation route from mercapturic acid to its sulfoxide was saturated.
- Oral administration of 1,3-D (50 mg/kg bw) produced significant alterations in non-protein sulfhydryl content in the liver, kidney, forestomach and glandular stomach of male rats. GSH conjugation is an important pathway for the depression of forestomach, glandular stomach, liver and kidney non-protein sulfhydryl content observed in this study suggesting that the ability of the rat to detoxify 1,3-D in this study may be compromised at an oral dosage level of ≥50 mg/kg.
- 1,3-D did not activate AhR, CAR, PXR or PPARα nuclear receptor in rat primary hepatocytes.
- The incidence of benign liver tumours (hepatocellular adenomas) was statistically increased in males and females by a trend test at 25 mg/kg bw/day. Pairwise comparison of tumour incidence, however, indicated a statistically significant increase only in male rats. The HCD from the Dow toxicology

laboratory demonstrates variable background incidences for hepatocellular adenomas in both male and female F344 rats. The liver tumor incidences in mid-dose males and high-dose females are within the relevant historical control ranges. In addition, the lack of statistical significance supports the observation that the incidences of benign adenomas in the mid-dose male and high-dose females were similar to those of controls. In terms of the incidence of hepatocellular adenomas in high dose males being just outside of the HCD range, employing Haseman's rule clarifies the lack of biological significance.

- Body weights were decreased as much as 15-16% and body weight gains were suppressed an average of 17-19% in rats ingesting 25 mg/kg bw/day Telone II.
- Possible neoplastic findings consisted of slightly increased incidence of benign submucosal mesenchymal tumours in the urinary bladder female CD-1 mice at 25 mg/kg bw/day (3/65) compared to controls (0/65). However, the mesenchymal tumours seen in three high-dose females were considered to be a secondary response to chronic irritation and represent a benign proliferation lesion indicating minimal or equivocal neoplastic activity. There was no increase in this tumour type in chronic studies with B6C3F1 mice up to a higher dose level of 50 mg/kg bw/day.
- Following inhalation exposure for 2-years to Telone II in B6C3F1 mice, the only tumorigenic response was an increased incidence of benign lung tumours (bronchioloalveolar adenoma) in male mice at 60 ppm (22/50 vs. 9/50 in controls). No treatment-related tumorigenic response was identified in males exposed to lower concentrations nor in female mice.
- No evidence of progression to malignancy for any tumour type observed.

Table 101: Compilation of factors to be taken into consideration in the hazard assessment

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
Rat Fischer 344	benign liver tumours (adenomas)	No	No	No	High-dose males only considering more extensive HCD data	Yes. significant reductions in body weight and body weight gain for high dose animals; mid-dose males within HCD	Oral	1,3-D did not activate AhR, CAR, PXR or PPARα nuclear receptor in rat primary hepatocytes
Mice B6C3F1	benign lung tumours (bronchioloalveolar adenomas) The incidence of 22/50 is only just outside the background incidence which goes up to 18/50.	No	No	No	Males only	Yes. Only observed at a dose level that exceeds the kinetically derived maximum dose (KMD) for 1,3-D in mice following inhalation exposure.	Inhalation	GSH depletion at dose levels that exceed the KMD
Mice CD-1	benign submucosal mesenchymal tumours in the urinary bladder	No	No	No	Females only	No	Oral	secondary response to chronic irritation and represent a benign proliferation lesion indicating minimal or equivocal neoplastic activity

10.9.2 Comparison with the CLP criteria

According to CLP criteria (Regulation (EC) No. 1272/2008) the hazard categories for carcinogens are as follows:

Table 82: Hazard categories for carcinogens

Category	Criteria	Evidence
1		
1A	Substances known to have carcinogenic potential for humans	Positive evidence from human epidemiological studies - human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen)
1B	Substances presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.	Animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen)
2	Substances which are suspected human carcinogens	The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.
Sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;		
Limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.		
Some important additional considerations for classification (a) tumour type and background incidence; (b) multi-site responses; (c) progression of lesions to malignancy; (d) reduced tumour latency; (e) whether responses are in single or both sexes; (f) whether responses are in a single species or several species; (g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity; (h) routes of exposure; (i) comparison of absorption, distribution, metabolism and excretion between test animals and humans; (j) the possibility of a confounding effect of excessive toxicity at test doses; (k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.		

- A statistically significant increase in incidence of benign liver tumours (adenomas) was observed in male Fischer 344 rats only. A statistically significant increase in the incidence of liver adenomas was not observed in female Fischer 344 rats, and there was no increase in liver adenomas observed in the chronic studies with mice. This increase was only observed at a dose level which also caused significant reductions in body weight and body weight gain and is considered to exceed the MTD for the chronic administration of 1,3-D in rats. The observed increase in incidence is only just outside the HCD range for this tumour type, there is no evidence for reduced latency nor progression to malignancy.

- A statistically significant increase in incidence of benign lung tumours (bronchioloalveolar adenomas) was observed in male B6C3F1 mice at 60 ppm (22/50 vs. 9/50 in controls). Bronchioloalveolar adenomas are a commonly observed spontaneous tumour in this strain of mice and more commonly observed in males. The incidence of 22/50 is only just outside the background incidence which goes up to 18/50. There was no increase in lung tumours in female B6C3F1mice, nor in Fischer 344 rats following chronic inhalation exposure to 1,3-D. The increased incidence in males was only observed at a dose level that exceeds the kinetically derived maximum dose (KMD) for 1,3-D in mice following inhalation exposure. There is no reduction in the tumour latency, they did not adversely affect survival of the animals, and there was no evidence of progression to malignancy.
- The slightly increased incidence of benign submucosal mesenchymal tumours in the urinary bladder of female CD-1 mice at 25 mg/kg bw/day (3/65) compared to controls (0/65) lacked statistical significance. Moreover, the mesenchymal tumours seen in three high-dose females were considered to be a secondary response to chronic irritation and represent a benign proliferation lesion indicating minimal or equivocal neoplastic activity. There was no increase of this tumour type in male CD-1 mice, nor in male or female B6C3F1 mice tested up to a higher dose level of 50 mg/kg/day, nor in Fischer 344 rats.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Not classified for carcinogenicity.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Not assessed in this dossier.

10.10.2 Adverse effects on development

Not assessed in this dossier.

10.10.3 Adverse effects on or via lactation

Not assessed in this dossier.

10.11 Specific target organ toxicity-single exposure

Table 103: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Acute oral toxicity study OECD TG 401 Sprague-Dawley CFY rats, 5/sex/dose GLP	Telone II 86/3293 (lot 0713054/062), with purity 97.2% of 1,3-Dichloropropene (cis- 1,3-dichloropropene content.≥45%), Oral route, doses of 75, 110, 170 and 250 mg/kg bw, Rat, SD, 5/sex/dose	Animals in all dose groups showed hunched posture and pilo-erection. 75 mg/kg bw: decreased respiratory rate in the males 110 mg/kg bw: decreased respiratory rate and lethargy, signs of ptosis and an isolated sign of diarrhoea 170 mg/kg bw: ataxia, ptosis and increased salivation, lethargy, decreased respiratory rate, ptosis, diarrhoea, diuresis, ataxia, tiptoe gait, red/brown staining around the snout, occasional body tremors, emaciation and pallor of the extremities 250 mg/kg bw: Increased lacrimation, diuresis LD_{50}: 150 mg/kg (110-170 mg/kg)	Anonymous 8 (1986), DAR, Spain, 2018
Acute oral toxicity study No guidelines followed; Sprague-Dawley CD SD rats (males only), 3/dose GLP	Telone II (Lot/Refer. No. UB1716284A, TSN105628). Purity: 97.2%; 51.4% cis- (Z-) and 45.8% trans- (E-) isomers; Groups of test animals were dosed in a split dosing regimen at doses (total) of 150, 225, 300 and 600 mg/kg using PEG 400 as a vehicle (phase 2) and another group dosed in a similar manner with rodent chow slurry (phase 3).	Phase 2 (see table 18 in section 10.1.1 for design of the study): Clinical observations in the treated animals consisted of various combinations of decreased activity, soft faeces, perineal soiling, perioral soiling, and/or eyelids partially closed. Two of the PEG 400 vehicle control animals also had clinical signs on day 1 that included perineal soiling and/or soft/watery faeces. Clinical signs in all animals had resolved by test day 3. All animals had gained weight by the end of the study. Gross pathological findings consisted of an ulcer or a thickened area in the nonglandular mucosa in the stomach in two animals at the 150 mg/kg bw/day dose level. All animals dosed with 300 mg/kg bw/day had gross pathological findings that included thickening and/or ulcers in the nonglandular mucosa of the stomach, and/or adhesions of the nonglandular serosa of the stomach to the spleen. There were no gross pathological findings in the 0 or 225 mg/kg bw/day group. All animals survived and gained weight by the end of 14-day observation period. Phase 3: Clinical signs in these animals consisted of soft faeces, perineal soiling, perioral soiling, and/or decreased activity that resolved by test day 3. These clinical signs were accompanied by reduced body weight gain on test day 2 compared to controls. Body weight gain was similar to controls by the end of the study. There were no gross pathological findings in the 300 mg/kg bw/day group. All animals dosed with 600 mg/kg bw/day in rodent chow slurry died by test day 2. Prior to death, clinical signs noted on day 1 included decreased activity, perioral soiling, perineal soiling, soft faeces, and/or periocular soiling. Gross pathological findings in the 600 mg/kg bw/day animals included watery contents in the GI tract, fluid in the thoracic cavity, facial and/or perineal soiling, congestion and dilatation of the stomach.	Anonymous 9 (2006) DAR, Spain, 2018
Acute oral toxicity study OECD TG 401 Sprague-Dawley CFY rats, 5/sex/dose GLP	Telone II microcapsules (Lot/Refer. No. M011608, TSN030336-0001). The purity of the test material was determined to have a 1,3 D loading of 29.1 ± 1.0(s)% by gas chromatography	All animals survived and appeared normal throughout the study and there were no gross pathological observations at test termination. The maximum dietary dose of 55.8 mg/kg bw had no effect on the survival of male rats following a single 2-hour dietary exposure	Anonymous 141 (2008)

Acute oral toxicity study OECD TG 401 Sprague-Dawley rats, 5/sex/dose GLP	Cis 1,3-dichloropropene (Batch No. 87/RM/716) Purity not available 75, 110, 170 and 250 mg/kg body weight administered as a solution in PEG 400	Major signs of toxicity noted in all dose groups 1 and 4 hrs after dosing were hunched posture, pilo-erection, lethargy, ptosis, and decreased respiratory rate/ Ataxia was noted in animals treated with 170 and 250 mg/kg at 1 and 4 hours after dosing. Common abnormalities noted at necropsy of decedents were abnormally red or hemorrhagic lungs, dark livers or patch pallor of the liver with haemorrhage and ulceration of the glandualr gastric mucosa. Blood vessels of the non-glandular gastric mucosa were prominent and the small intestine appeared haemorragic. Necropsy of surviving animals revealed large areas of white foci or multiple white foci of the region (organ not specified). Surviving males treated with 100 mg/kg showed adherence of stomach to the liver. LD_{50} – all animals – 121 (107 – 137) mg/kg bw	Anonymous 17 (1988)
Acute oral toxicity study EEC Method B1 Fischer F344 rats, 5/sex/dose GLP	Trans-1,3-Dichloropropene Batch No. TR88001 Purity 96.7% 75, 90, 120 and 160 mg/kg administered undiluted	Signs of toxicity related to dose levels: the principle signs of reaction to treatment were diarrhoea or voiding of soft faeces, cyanosis, increased lachrymation, a hunched posture, lethargy, piloerection and ante-mortem prostration. Other clinical signs included abasia, increase salivation and ataxia among rats does at 90 mg/kg and above and an isolated case of ante-mortem hypothermia. Piloerection, a hunched posture and an unkempt appearance were commonly apparent on day 2 and day 3 among rats treated at the low or intermediate dose-levels. The recovery of rats surviving the toxic effects of trans-1,3-dichloroproperne was generally advanced by day 4 although the unkempt appearance of many rats persisted for up to nine days after treatment. Effects on organs: necropsy of decedents revealed similar abnormalities at each dose level. Common pathological changes included discoloration of the renal medulla, congestions of the lungs, darkening of the liver, the presence of dark or light patches on the liver, exaggeration of the hepatic lobular pattern, petechiae on thymus and liquid contents and/or inflammation of the stomach. Other isolated findings included heamorrhage of the stomach, heamorrhage or inflammation of the caecuum, pallor of the spleen and pale, raised areas on the kidneys. LD_{50} – all animals – 94 (82 – 108) mg/kg bw	Anonymous 18 (1988)
Acute oral toxicity study OECD TG 401 Fischer F344 rats, 5/sex/dose GLP	Cis-1-3-Dichloropropene Batch No. ST88/253 Purity 96.9% 38, 68, 90, 120, 160, and 213 mg/kg, administered undiluted	Common signs of reaction to treatment observed among rats dosed at 38, 51, or 68 mg/kg were limited to voiding of soft faeces or diarrhoea within 4 hrs of dosing and piloerection and/or unkempt appearance during day 2. Recovery of surviving rats was complete by day 3. Only three rats survived treatment at 90 mg/kg and above. The majority of deaths occurred within four hours of dosing. Principal clinical signs observed prior to death were increased lachrymation and salivation, abasia or ataxia and diarrhoea. Less commonly lethargy, cyanosis and unkempt appearance were observed and there were isolated cases of hypothermia, piloerection and hunched posture. All surviving rats had gained weight relative to their Day 1 bodyweights by the end of the 14-day observation period. The principal macroscopic abnormalities found in decedents were abnormal contents, inflammation and haemorrhage of the stomach, darkening of the liver, lung congestion and discolouration of the renal medulla. No macroscopic abnormalities were found in rats that survived treatment LD_{50} – all animals – 85 (68 – 113) mg/kg bw	Anonymous 23 (1989)

Acute dermal toxicity study OECD TG 402 SD CFY rats, 5/sex/dose GLP	Telone II 86/3293 containing 97.2% 1,3-Dichloropropene dermal dose of test material (occluded application) at doses of 500, 800, 1300 or 2000 mg/kg bw	Clinical observations: lethargy in all animals on the day of dosing. Dose related signs of toxicity (decreased respiratory rate, increased lacrimation, increased salivation, ataxia, red/brown staining around the snout or mouth) were observed from 1300 mg/kg; signs of skin irritation manifested by edema, eschar formation or possible subcutaneous haemorrhage were seen at the test site. The signs of toxicity appeared earlier in animals treated with 2000 mg/Kg, disappear on day two in the animals treated with 500 mg/Kg and on day three no systemic abnormalities in all of the surviving animals were noted. The body-weight gains of the majority of animals in this study were considered to be within normal limits. The abnormalities seen at necopsy of animals were associated with the lungs, liver, gastrointestinal tract and subcutaneous tissues at the site of application, they appeared haemorrhaged. LD_{50} all animals: 1200mg/kg bw	Anonymous 10 (1986) DAR, Spain, 2018
Acute dermal toxicity study EPA 81-2 Guideline NZW rabbits, 5/sex/dose GLP	AGR 233011 - lot TB 860825-5), a liquid formulation with 52,63% w/w cis-1,3-Dichloropropene(Z) and 44,91% w/w trans-1,3,-D(E) 200, 1000 mg/kg bw, (occluded application) time of exposure: 24 hours.	Clinical observations: some rabbits of both dose groups were restless and squealed shortly after treatment; all rabbits given 1000 mg/Kg were lethargic prior to death. All animals had skin irritation (edema, erythema and necrosis) at the dermal test site. No significant effects on body weights were observed. At necropsy all rabbits had skin irritation with subcutaneous haemorrhage, necrosis, erythema, edema and/or crusts. LD50 all animals: 333 mg/kg bw	Anonymous 11 (1987) DAR, Spain, 2018
Acute dermal toxicity study OECD 402 SD CFY rats, 5/sex/dose GLP	Cis 1,3-dichloropropene (Batch No. 87/RM/716) 500, 800, 1300 or 2000 mg/kg bw (occluded application)	Survinving animals showed signs of toxicity 1 day after dosing, including hunched posture, pilo-erection, lethargy, decreased respiratory rate and ptosis. Isolated incidents of ataxia were noted in animlas treated with 1300 mg/kg or greater. Signs of skin irritation characterised by edema, eschar or hardening of the treatment site were noted during the study periold. The eschar formation persisted in surviving annimals until termination of the study. A small loss in body weight was noted in one female treated with 800 mg/kg over the first week. Expected gains in body weight were noted in all surving animals. LD_{50} –All animals – 794 mg/kg bw	Anonymous 19 (1988)
Acute dermal toxicity study OECD 402 Fischer F344 rats, 5/sex/dose GLP	Trans-1,3-Dichloropropene Batch No. TR88001 Purity 96.7% 488, 781, 1250 and 2000 mg/kg of undiluted test material (semiocclusive application))	Signs of toxicity related to dose levels: Principle signs of reaction to the treatment observed within 5 hours of topical application were voiding of soft faeces or diarrhoea, lethargy and, particularly at the high dose levels, increased lachrymation. On the following day ataxia or abasia occurred among rats dosed at 1250 or 2000 mg/kg, voiding of soft faeces or diarrhoea persisted among those treated at 488 or 781 mg/kg and animals at all dose-levels showed an unkempt appearance and, less commonly, were prostrate. Two rats found with hind-limb mobility on day 3 were sacrificed on humans grounds. Effects on organs: Gastro-intestinal irritation and petechiae on the thymis. One female rat killed on day 14 showed local irritation of the stomach. No other macroscopic abnormalities abnormalities were found during necropsy of the rats that survived treatment. Necropsy of decedents revealed similar macroscopic abnormalities at each dose level. Common pathological changes included fluid contents, inflamation and/or heamorrhage of the stomach, petechiae on the thymus and fluid contents of the intestinal tract. Other isolated findings included exaggerated hepatic lobular pattern, dark spleen, pallor of the kidneys and the presence of blood in the urine. LD_{50} –All animals – 1575 mg/kg bw	Anonymous 18 (1988)

Acute dermal toxicity study EEC Method B3 Fischer F344 rats, 5/sex/dose GLP	cis-1,3-dichloropropene (undiluted) at doses of 400, 560, 784, 1098 and 1537 mg/kg (occluded application)	Systemic toxicity was observed at 400 mg/kg other than the death of a single death on day 3. Voiding of soft feces and lethargy were common among rats given higher dose levels and a few cases of unkempt appearance. hypothermia, abasia, ataxia, hunched back or bloody discharge from the nose in groups dosed at 784 mg/kg and above. Rats that died showed no ante-mortem clinical changes, nor did those surving treatment. Recovery of rats surving treatment was generally advanced by day 3 but remained incomplete until day 6. Sites of application commonly developed a slight erythema or erythema that persisted upt o 4 days after removal of patch. Oedema was apparent among rats treated at 1537 mg/kg. Despite loss of body weight during the first week of observation, all surviving rats gained wieght relative to their day 1 body weight on day 14. LD_{50} –All animals – 1090 mg/kg bw	Anonymous 23 (1989)
Acute inhalation toxicity study OECD 403 Wistar albino rats, 5/sex/dose GLP	Telone II (Lot 0713054/062) containing 98,4% 1,3-Dichloropropene, Whole-body exposure for 4 hours to vapour concentrations of 0, 1.62, 2.64, 2.70 or 3.07 mg/l.	During the exposure to the test substance, all animals showed partial closing of the eyes, lacrimation, reduce respiratory rate and irregular respiratory movements. At the observation period, hunched posture and restless behaviour were seen in the majority of exposed rats. Clinical signs persisted in a proportion of rats for several days following exposure (lethargy, reduced respiratory rate, shallow respiratory movements, irregular respiratory movements, diarrhoea, brown staining of the fur and fur loss). The body weights and food and water consumption of survivors were reduced for up to 6 days following exposure. Finding of necropsy of surviving rats were associated fundamentally with the lungs and respiratory tract, gastrointestinal tract and adrenals. The lung weight to body weight ratio were higher in the decedents and for two survivors. LC_{50} all animals - between of 2.70 and 3.07 mg/L	Anonymous 12 (1987) DAR, Spain, 2018
Acute inhalation toxicity study EPA guidelines with no deviations from OECD 403 (1981) Fischer 344 rats, 5/sex/dose GLP	Telone II (AGR 233011), containing 52.6% cis-1,3-D and 44.9% trans-1,3-D Whole-body exposure for 4 hours to vapour concentrations (analytical) of 3.88, 4.10, 4.70 mg/l.	All animals showed signs of irritation and were lethargic at all dose levels during and after exposure. Body weights of survivors decreased during the first week post-exposure and increased during the second week. Animals which died as a result of exposure had facial soiling and/or haemorrhages in multiple lung lobes. Surviving animals did not have any exposure-related gross observations. LC_{50} all animals - between of 3.88 and 4.7 mg/L	Anonymous 13 (1987) DAR, Spain, July 2018
Acute inhalation toxicity study OECD 403 Fischer F344 rats, 5 male /dose GLP-not stated	TELONE II (Lot 121006-9) contained 97.5% 1,3-D (cis and trans). Nose-only exposure to a nominal concentration of 14,.980 ppm (analytical concentration 14000ppm) TELONE II vapours for 1-hour	Clinical effects noted during the one-hour exposure period included perinasal soiling and slow, labored breathing in all animals. Post-exposure observations on test day 1 included mortality; inability to walk; slow, shallow, noisy, labored, and/or deep respiration; perinasal, perineal, and/or abdominal soiling; bluish skin and mucous membranes; and partially closed eyelids. Decreases in resistance to removal, muscle tone, extensor-thrust response, reactivity to stimuli and responsiveness to touch were also observed post-exposure on test day 1. Necropsy findings included mottled lungs and serosanguineous soiling of the muzzle in all animals, and multifocal ulcers of the stomach in one animal. One-hour inhalation exposure to 64 mg/L (15.90mg/L – concentration estimated for 4h exposure) TELONE II caused mortality in all exposed animals	Anonymous 142 (2003); DAR, Spain, 2018

Acute inhalation toxicity study OECD 403 Fischer F344 rats, 5/sex/dose GLP	cis 1,3-Dichloropropene, Purity: 95.6% cis, 1.5% trans, 0.2% 1,2-dichloropropane Whole body exposure for 4 hours to vapour concentrations (analytical) of 3.88, 4.10, 4.70 mg/l.	Clinical signs: all animals exposed to 2.6, 3.5, or 4.63 mg/L had labored breathing, at the lowest concentration, the labored breathing was reversible and not observed 2 h after exposure. Several animals exposed to 2.6 mg/L were noted to have eye irritation during the exposure which was also readily reversible. Several animals exposed to 3.5 had reddish material, most likely porphyrins around the eyes and nose on the day after exposure; reddish material was not noticed at day 7. Gross pathology: 5 animals exposed to 1020 ppm had corneal opacities noted at necropsy. Pulmonary edema was noted in all rats exposed to 1020 ppm and 3 animals exposed to 771 ppm. 2 animals exposed to 771 ppm exhbited hydrotorax, and several animals exhibited facial soiling and perineal soiling. There were no grossly visible lesions noted in animals surving until the end of the 2-wk post-exposure period.	Anonymous 20 (1990)
Acute inhalation toxicity study OECD 403 Sprague-Dawley Crl:CD(SD)BR rats, 5/sex/dose GLP	trans-1,3-dichloropropene (Lot no. ST 88/098), purity – 96.7% Head only exposure for 4 hours to aerosol concentrations of 3.859, 4.449, 4.98, 6.36 mg/l.	Signs of toxicity related to dose levels: 3.859 mg/l: on days 2 and 3 all animals exhibited piloerection, lethargy and abnormal fur. One female exhibited cold/hunched posture and loss of condition on day 5 and/or 6. Two animals died on days 4 and 6. 4.449 mg/l: all animals showed piloerection and lethargy on days 2 to 6 and wet or stained fur on days 2 to 3. One animal died on day 8. 4.980 mg/l: piloerection and lethargy in four males and three females on days 2, 3 and/or 4. Prostration, tremors, ataxia and posterior paresis in two males on day 4. Tremors and ataxia in one female on days 4 and 5. Abnormal fur in four males and three females on days 2 and 3 and/or 4 and 5. Three animals died on day 2, two on day 4 and one on day 5. 6.360 mg/l: piloerection in three males and two females on day 2. Tremors and ataxia in one female on day 2. Wet or stained fur in three males on days 2 and 3, and in two females on day 2. Seven animals died on day 7 and one on day 4. Gross pathology: Effects on organs: The most consistent necropsy findings were dark/red and inflated lungs suggestive of local effects on the respiratory tract. Yellow/dark contents were also found in the stomach of five animals, and the kidneys of two decedents were pale; possibly suggesting effects outwith the respiratory tract. Most treated animals surviving to study termination were either unremarkable at necropsy or had minor lesions similar to those of the controls. One dose group 4.449 mg/l female had a pale zone in the corticomedullary region in the kidney. Taken together with the pale kidneys in two of the decedents, this was also suggestive of effects on the kidneys. The occurrence of nephrotoxicity histologically and the occurrence of delayed deaths after exposure suggested kidney failure was the primary cause of death. Congestion and oedema in decedents suggested cardiopulmonary failure was the proximal cause of death. There was a cellular response in the lungs of three individuals suggesting the test substance may have also had a local effect.	Anonymous 21 (1989)
2-week vapor inhalation toxicity study in Fischer 344 rats, 15/sex/dose Non-guideline study GLP	Cis-1,3-Dichloropropene 95.6%; Lot No. 2047 Whole body exposure to vapour, 6h/d, 5d/wk, 9 exposures, Concentrations: 0, 10, 60, or 150 ppm (0.0, 0.05, 0.27 or 0.68 mg/liter)	All animals survived, rats exposed to 150 ppm appeared lethargic during first exposure but not subsequent expsoures; other clincial data showed no effect. Histopathologic examination revealed changes of moderate severity in the respiratory and olfactory mucosa in the nasal cavity (moderate bilateral hyperplasia of the respiratory epithelium, moderate bilaterl dgeneration of the olfactory epithelium and exudate in nasal passages) of males and female rats exposed to 150 ppm cis-1,3-D; there were no exposure-related histopathologic changes in animals in the two lower exposure groups. Male and female rats exposed to 150 ppm cis-1,3-D lost weight during the course of the two-week study. Body weights of male and female rats exposed to 60 ppm were slightly decreased (3%) from control values at the end of the study. The no-adverse-effect-level (NOAEL) was 60 ppm.	Anonymous 48 (1990)

sub-chronic inhalation toxicity study, 13-weeks in Fischer 344rats, 10/sex/dose OECD 413 Minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of the relevant results. GLP	Cis-1,3-Dichloropropene 95.6%; Lot No. 2047 Whole body exposure to vapour, 6h/d, 5d/wk, for 13 weeks, Concentrations: 0, 10, 30, or 90 ppm (0.0, 0.05, 0.14 or 0.41 mg/liter)	All animals survived to the end of the study with no clinical observations related to exposure to the test materials. Exposure-related microscopic changes occurred only in the nasal cavities of male and female rats exposed to 90 ppm; changes consisted of bilateral degeneration of olfactory epithelium and bilateral multifocal hyperplasia of the respiratory epithelium. Body weights of male rats were significantly decreased from control values throughout the 13 week exposure period (decreased 8% from control values at the end of the study). The no-adverse-effect-level (NOAEL) was 30 ppm	Anonymous 49 (1991)
2-week vapor inhalation toxicity study in Wistar rats, 5/sex/dose GLP	Trans-1,3-Dichloropropene Batch No. A4308 05008, Purity 96.7% Nose only exposure to vapour, 6h/d, 5d/wk, for 14 days, Concentrations: 0, 30, 100 or 300 ppm (0.0, 0.14, 0.45 or 1.36 mg/liter)	During exposure, changes in breathing pattern were observed such as superficial breathing at a visually reduced frequency in animals of the mid and high concentration group on the first exposure day, and a slightly reduced breathing frequency in animals of the mid and high concentration on most of the other exposure days. No abnormalities were observed after exposure. Individual observations in the mornings revealed sparsely haired skin in 3 out of 5 males and all 5 females of the mid and high concentration group. All animals survived until scheduled necropsy. A concentration-related decrease in body weight gain was observed in male rats. A concentration-related increase in relative lung weight was observed in males of the mid and high concentration group. Females of the high concentration group also showed a significantly increased relative lung weight. Statistically significant increases were further observed in relative weights of the adrenals (males and females of the high concentration group), kidneys (males and females of the high concentration group), brain (males and females of the high concentration group and males of the mid concentration group), and head (males of the mid concentration group).	Anonymous 50 (1999)
sub-chronic inhalation toxicity study, 13-weeks in Wistar rats, 10/sex/dose OECD 413 GLP	Trans-1,3-Dichloropropene Batch No. A4308 05008, Purity 96.7% Nose only exposure to vapour, 6h/d, 5d/wk, for 13 weeks, Concentrations: 0, 10, 30, or 90 ppm (0.0, 0.05, 0.14 or 0.41 mg/liter)	During exposure, no changes in breathing pattern or behaviour were observed. No abnormalities were observed after exposure. Individual daily observations prior to exposure revealed sparsely haired skin in females of the mid and high concentration group. A decrease in body weight gain was observed in male and female rats of the mid and high concentration groups. A decrease in food intake was seen in animals of the high concentration group. Food conversion efficiency was also decreased in males of this group. In plasma obtained from animals of the high concentration main study group, increases in alanine aminotransferase, triglyceride (females only) and in the albumin/globulin ration (males only) were found. An increase in urinary volume and a tendency towards a decreased urinary density were found in female animals of the high concentration main study group. Relative weight of the liver was increased in male and female animals of the high concentration main study group and in females of the high concentration interim kill group. Relative weight of the kidneys of females of the high concentration main study group was also increased. No treatment-related gross abnormalities at necropsy, nor histopathological changes at microscopic examination of organs and tissues were found. In the low concentration group, no effects were seen that were considered of toxicological significance. The no-adverse-effect-level (NOAEL) was 8.9 ppm	Anonymous 51 (1999)

Summary of human data on STOT SE

Human data (ref. DAR, Spain, 2018, Vol. 3 B.6.9 (AS))

OBSERVATIONS BEFORE 2002:

In the USA since January 1997 there have been 22 reports of alleged human health effects associated with 1,3-D reported to the U.S. EPA by Dow AgroSciences. These allegations were received from PROSAR, a human health poison control center, employees of Dow AgroSciences, customers, and other sources. Two of the incidents were litigation-related; one alleges nasal bleeding and blistering following accidental exposure to the face. The other litigation-related case involves allegations of a wide variety of non-specific symptoms in individuals who were engaged in farming some land near and adjacent to a farm where pesticides had been applied; the alleged symptoms include pain, discomfort, anxiety, fear, anguish, distress, inconvenience, disability, diminished quality and enjoyment of life, coughing, shortness of breath, headaches, dizziness, disorientation, nausea, vomiting, fatigue, itchy watery eyes, abnormal liver function. The most common incidents (9) involved allegations of eye irritation, burning or watering eyes, or corneal burn. Several incidents involved allegations of skin irritation or burns from direct dermal contact with 1,3-D, and several involved allegations of a variety of symptoms possibly related to vapor drift from adjacent application sites including diarrhea, vomiting, nausea, headache, nasal irritation, dizziness, tingling, lightheadedness. One incident involved allegations of exposure to leaking container of product transported improperly in enclosed van, with alleged symptoms of adult respiratory distress syndrome, coagulopathy, liver damage, kidney damage, gastrointestinal bleeding, confusion, hallucinations/delusions, elevated creatinine, hepato-renal syndrome, coma.

Pesticide-related illnesses have been tracked within the state of California for nearly 50 years. The California Environmental Protection Agency, Department of Pesticide Regulation (DPR) maintains a surveillance program which records human health effects of pesticide exposure. From the review of California data on suspected 1,3- D poisonings, it appears that a majority of incidents involved illnesses or injuries to workers who applied 1,3-D as a soil fumigant in fields. A large proportion of the cases occurred when workers were preparing, operating, cleaning, or repairing application equipment; however, label changes since 1992 have been adopted which may have prevented reported exposures. Some individuals with inhalation exposures have reported symptoms such as headache, chest pain, fatigue, irritability or difficulty concentrating, persisting for as long as two years after initial exposure. Accidental ingestion of 1,3-D has led to one reported fatality in a 27 year old previously healthy worker; he developed gastrointestinal distress, adult respiratory distress syndrome, hematological and multiorgan failure including pancreatic damage, hepatorenal function impairment and died after 40 hours (Hernandez et al, 1994). Dyspnea may be reported after inhalation exposure (IARC, 1986). In a group of 80 people exposed to dichloropropene because of a truck spill, 4 complained of chest discomfort. In a group of 41 people who had liver enzymes levels assayed after being exposed to dichloropropene because of the truck spill, 11 had slightly elevated SGOT and/or SGPT levels (Hathaway et al, 1996). Five of 28 patients interviewed 12 weeks after exposure complained of chest discomfort, one of who was diagnosed with pneumonia. Smarting of the skin and first-degree burns may occur with short-term exposure; second-degree burns may be seen after prolonged contact (CHRIS, 1999). Irritation of the mucous membranes may be noted (IARC, 1986; Grant & Schuman, 1993). Sensitization occurred in a 20-year-old man. Erythematous, pruritic papular lesions developed on the hands and feet. The lesions disappeared after topical corticosteroid therapy. Patch testing was positive (van Joost & de Jong, 1988).

OBSERVATIONS SINCE 2002:

DowAgrosciences contracts SafetyCall International for immediate response to acute exposure reporting from the general public or healthcare providers. A search of SafetyCall International's reporting database (part of AERC database) from January 1st 2002 to date revealed 67 reports of exposure incidents, primarily in the USA and Canada.

Forty one (41) of these were classified by SafetyCall International's toxicologists as "mild" and 4 "asymptomatic". Typically these involved mainly irritation of skin, eye, throat, nausea and headache – either in isolation or combination.

Twenty two (22) were classified by SafetyCall International's toxicologists as "moderate", and also included many skin, eye, throat irritations of a more significant nature, along with tight chest, chest pain,

dyspnoea/shortness of breath, dizziness, tachycardia and incoordination – again in isolation or in combination. The remaining 4 reports were not considered to be product-related.

None was classified by SafetyCall International's toxicologists as "severe" and there were no documented fatalities or long term adverse health effects after the acute exposures, although longer term follow up is limited.

DowAgrosciences collects reports on its AERC from Dow Health Services Staff and employees, through to end users and the general public. Eighty eight (88) reports of exposure incidents since 2002 are documented – some relate to small group exposures, e.g. families.

Thirty six (36) of these appear to be duplicates (the same cases) of those reported by SafetyCall International, which is to be expected.

Of the remainder, the vast majority (21) were reports of skin and/or eye irritation, along with 4 to 5 each of respiratory irritation, gastro-intestinal irritation, and headaches. There were single reports of flushing, nosebleeds, joint pains/tremor and leg cramps. Four (4) cases were asymptomatic.

Included in the data are litigation cases dating back to a 1975 exposure incident in Yuba City, California – following a truck spill and clean-up operation – 16 claims were originally launched, including one allegation of sarcoidosis, and one allegation of death due to testicular cancer. The cases were defended successfully.

1,3-Dichloropropene: Occupational Illness and Injury Reports – California Department of Pesticide Programs (Anonymous 144, 2015)

In California between 1982-1990, 51 cases were reported to the California Department of Pesticide Regulation's Pesticide Illness Surveillance Program (PISP), which maintains a database of pesticide-related illnesses and injuries occurring in California (Sanborn and Powell, 1994). Case reports are received from physicians and through workers' compensation records. Of these 51 cases, the health effects attributed to exposure to 1,3-D alone, or in combination with other pesticides, were rated as definite (33 cases), probable (9 cases) or possible (9 cases). The health effects involved were systemic (16 cases), eye (14), skin (18), and combined eye-skin effects (3). From 1990 to 1995, 1,3-D use in California was suspended. As a result, from 1990 to 1997, there were no reports of illnesses associated with 1,3-D applications. In 1998, PISP identified one possible case involving 1,3-D alone with no other cases appearing until 2002. Then, in the 10 years from 2002 to 2011, the PISP identified 17 exposure episodes that gave rise to 71 cases associated with 1,3-D either alone or in combination with chloropicrin (Figure 4) (Anonymous 144, 2015). The 71 cases were classified as 1 definite, 54 probable and 16 possible.

The single case reported as "definite" in 2011 included a formulation that contained both 1,3-D and chloropicrin. An employee of a soil fumigant manufacture/supplier connected a hose to an empty cylinder to purge it of any remaining chemical. The hose fell and fumigant liquid splashed into his right eye. Redness and eye irritation were reported, as well as blurred vision and burning sensation.

Of the 72 recently reported cases (i.e., between 1998 and 2011), there were 5 cases with 1,3-D used alone between 1998 and 2011. Four of these cases exhibited respiratory symptoms. In 2007, 1 episode involving 3 cases was reported. Three mechanics were working on the air conditioning system of a fumigant tractor last used 50 days prior. The tractor was reported as dirty and each mechanic became ill after working on the tractor inside the shop. One reported chest tightness, where the other 2 reported upper respiratory tract irritation, irritated and watery eyes, dizziness, and runny nose.

10.11.1 Short summary and relevance of the provided information on specific target organ toxicity-single exposure

In the acute oral studies , dermal and inhalation studies, the common clinical symptoms were body weight loss, hunched posture, pilo-erection, lethargy, decreased respiratory rate, signs of ptosis, diarrhoea, decreased activity, soft faeces, perineal soiling, perioral soiling, lacrimation, palpebral closure, facial soiling,
In the acute inhalation study (Anonymous 12, 1987) clinical signs persisted in rats for several days following exposure (lethargy, reduced respiratory rate, shallow respiratory movements, irregular respiratory movements,

diarrhoea, brown staining of the fur and fur loss). Finding of necropsy of surviving rats were associated fundamentally with the lungs and respiratory tract, gastrointestinal tract and adrenals. The lung weight to body weight ratio were higher in the decedents and for two survivors.

In 2-week and 13-week sub-chronic studies conducted via the inhalation route there was evidence of degenerative changes in the nasal olfactory epithelium and histopathological changes of the respiratory epithelium in rats after subchronic inhalation exposure. Histopathological findings in the non-glandular stomach and generalized systemic toxic effects were also observed.

Epidemiological data of workers exposed to 1,3-D revealed health effects involved eye and/or skin irritation respiratory symptoms (upper respiratory tract irritation, irritated and watery eyes, dizziness, and runny nose), a variety of symptoms possibly related to vapor drift from adjacent application sites including diarrhea, vomiting, nausea, headache, nasal irritation, chest pain, dizziness, tingling, lightheadedness, pain, discomfort, anxiety, fear, anguish, distress, inconvenience, disability, diminished quality and enjoyment of life, irritability or difficulty concentrating, coughing, shortness of breath, disorientation, fatigue, itchy watery eyes, abnormal liver function.

10.11.2 Comparison with the CLP criteria

Classification as either STOT-SE 1 or 2 is applicable to substances that have produced non-lethal toxicity in humans, or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant non-lethal toxicity in humans following a single exposure.

Evidence from human incidents described in DAR, 2018 and Anonymous 144, 2015 is restricted to reports of adverse health consequence, with uncertainty about exposure conditions, and not provide the scientific details.

Classification as STOT-SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

In the CLP Regulation, paragraph 3.8.2.2.1 (a) it is stated that: “Respiratory irritant effects (characterised by localised redness, oedema, pruritis and/or pain) that impair function with symptoms such as cough, pain, choking, and breathing difficulties are included. This evaluation will be based primarily on human data”. Epidemiological data of workers exposed to products containing 1,3-D have revealed respiratory symptoms such as upper respiratory tract irritation, runny nose, nasal irritation, chest pain. In the CLP Regulation, paragraph 3.8.2.2.1 (d) it is stated that: “There are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation”.

In the acute inhalation study (Anonymous 12, 1987) finding of necropsy of surviving rats were associated fundamentally with the lungs and respiratory tract. In Anonymous 20 (1990) study all animals had labored breathing, at the lowest (2.6 mg/L, non-lethal) concentration, the labored breathing was reversible and not observed 2 h after exposure. In addition, in the 2-week inhalation study in rats (Anonymous 48, 1990) histopathologic examination revealed changes of moderate severity in the respiratory and olfactory mucosa in the nasal cavity (moderate bilateral hyperplasia of the respiratory epithelium, moderate bilaterl dgeneration of the olfactory epithelium and exudate in nasal passages); in 13-weeks inhalation study in rats (Anonymous 49, 1991) exposure-related microscopic changes occurred in the nasal cavities of male and female rats; changes consisted of bilateral degeneration of olfactory epithelium and bilateral multifocal hyperplasia of the respiratory epithelium. These findings are considered relevant as part of weight of evidence evaluation of the potential of 1,3-D to cause respiratory tract irritation.

Respiratory irritation observed both in animal studies and human epidemiological data only occur in the absence of other more severe effects in the respiratory system, fulfilling the CLP criterion described in paragraph 3.8.2.2.1 (e): “this special classification would occur only when more severe organ effects including in the respiratory system are not observed”.

Considering all the available data, it is considered that criteria (a), (d) and (e) described in paragraph 3.8.2.2.1 of the CLP for respiratory tract irritation are fulfilled.

Epidemiological data of workers exposed to 1,3-D revealed health effects involved dizziness, vomiting, nausea, headache, tingling, light headedness, pain, discomfort, anxiety, fear, anguish, distress, inconvenience, disability, diminished quality and enjoyment of life, irritability or difficulty concentrating, disorientation, fatigue.
In the acute oral studies , dermal and inhalation studies, the common clinical symptoms were body weight loss, hunched posture, pilo-erection, lethargy, decreased respiratory rate, signs of ptosis, diarrhoea, decreased activity, soft faeces, perineal soiling, perioral soiling, lacrimation, palpebral closure, facial soiling,
In the acute inhalation study (Anonymous 12, 1987) clinical signs persisted in rats for several days following exposure (lethargy, reduced respiratory rate, shallow respiratory movements, irregular respiratory movements, diarrhoea, brown staining of the fur and fur loss).

According to Regulation (EC) No. 1272/2008 (the CLP Regulation) the criteria for classifying substances as Category 3 (transient target organ effects) for STOT SE narcotic effects are:

(a) central nervous system depression including narcotic effects in humans such as drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination, and vertigo are included. These effects can also be manifested as severe headache or nausea, and can lead to reduced judgment, dizziness, irritability, fatigue, impaired memory function, deficits in perception and coordination, reaction time, or sleepiness.

(b) narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure.

Substances that meet these criteria would require classification in Specific-Target Organ Toxicity – Single Exposure (STOT-SE), Category 3 – H336 - May cause drowsiness or dizziness

The evaluation of available experimental data on 1,3-D (mix of isomers), cis 1,3-D (cis isomer) and trans 1,3-D (trans isomer) indicates that these substances have a comparable profile with respect to the potential for Specific-Target Organ Toxicity – Single Exposure (STOT-SE), Category 3 – H336 - May cause drowsiness or dizziness.

10.11.3 Conclusion on classification and labelling for STOT SE

Classification for specific target organ toxicity after single exposure in category 3 (STOT SE 3) with statement H335 “May cause respiratory irritation” and H336: ‘May cause drowsiness or dizziness’ is justified.

10.12 Specific target organ toxicity-repeated exposure

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

Table 104: Summary table of animal studies on repeated oral dose toxicity study

Method	Results	Remarks	Reference
Rat studies			
A CHRONIC TOXICITY AND ONCOGENICITY STUDY WITH DD-92 IN THE RAT VIA ORAL GAVAGE ADMINISTRATION 94.8% purity DD-92 was previously a tradename for 1,3-D; No information on isomer composition is available in the study report. Sprague Dawley CR® rats 0, 2, 10 and 25 mg/kg bw/day OECD TG 453 GLP	NOAEL: Chronic 2 mg/kg/day Oncogenic 25 mg/kg bw/day LOAEL: Chronic: 10 mg/kg/day Chronic NOAEL: *2 mg/kg/day based on 10 mg/kg/day findings of* increased incidence and/or severity of hyperplasia and hyperkeratosis of the stratified squamous epithelium lining the forestomach. **Not carcinogenic**	This study is acceptable with some reservations. Deviations with respect to OECD TG 453: the group sacrificed at 12 months should have contained 20 animals/sex/group instead of the 10 used in this study. For haematology, 20 animals/sex/group should have been examined instead of 10; in addition, an analysis at three months should have been performed. The same is valid for urinalysis.	Anonymous 53, 1998
Telone II soil fumigant: 13-week dietary toxicity and 4-week recovery studies in Fischer 344 rats Dietary (microencapsulated) 5, 15, 50, 100 mg/kg/day OECD TG 408 GLP Purity 96.0%	NOAEL: 5 mg/kg/day LOAEL: 15 mg/kg/day Hyperkeratosis and basal cell hyperplasia of the non-glandular mucosa of the stomach	This study is acceptable with respect to OECD TG 408	Anonymous 146, 1993
TELONE II SOIL FUMIGANT: **TWO-YEAR** CHRONIC TOXICITY /ONCOGENICITY STUDY IN FISCHER 344 RATS Dietary (microencapsulated) 0, 2.5, 12.5 and 25 mg/ kg/ day OECD TG 453 GLP Purity 96.0% 50.7% cis 45.1% trans	NOAEL: Chronic: 2.5 mg/kg/day Oncogenic: 2.5 mg/kg/day LOAEL: Chronic: 12.5 mg/kg/day Oncogenic: 12.5 mg/kg/day Chronic LOAEL: Depression of b.w/b.w.g. in male, day 92 onwards Foci of altered cells in the liver. Basal cell hyperplasia of the non-glandular mucosa of the stomach. Carcinogenic LOAEL: **Increase incidence of benign liver tumours (hepatocellular adenomas)**	This study is acceptable with some reservations. Deviations with respect to OECD TG 453: the group sacrificed at 12 months should have contained 20 animals/sex/group instead of the 10 used in this study. For haematology, 20 animals/sex/group should have been examined instead of 10; in addition, an analysis at three months should have been performed. An urinalysis at three months should have been performed.	Anonymous 54, 1995

National Toxicology Program (NTP). (1985) Toxicology and carcinogenesis studies of Telone II® (technical-grade 1,3-dichloropropene containing 1% epichlorohydrin as a stabilizer) in F344/N rats and B6C3F1 mice (gavage studies). U.S. Department of Health and Human Services Technical Report Series No. 269 F344 rats – 0, 25 and 50 mg/kg OECD Guideline – TG 451 GLP – yes	On the basis of forestomach and liver neoplasms in rats, the LOAEL for cancer in the study is 21.4 mg/kg (50 mg/kg/day × 3 days/7 days) and the NOAEL for rats is 10.7 mg/kg (25 mg/kg/day × 3 days/7 days)	Not acceptable Deviations with respect to OECD TG 451 – dosing 3 times in a week and epichlorohydrin – a stabilizer that was used in Telone II may be partially responsible for the hyperplasia and squamous cell papilloma/carcinoma in the rat forestomach	In: TOXICOLOGICAL REVIEW OF 1,3-DICHLOROPROPENE (CAS No. 542-75-6) - In Support of Summary Information on the Integrated Risk Information System (IRIS), *May 2000* - EPA/635/R-00/001 Draft Assessment Report, Spain, 2018
Mouse studies			
Oncogenicity Study with DD-92 in the Mouse via **Oral** Gavage Administration Albino mouse **CD®-1** 0, 2, 10 and 25 mg/kg/day OECD TG 451 GLP DD-92 was previously a tradename for 1,3-D; No information on isomer composition is available in the study report.	NOAEL: Chronic: 10 mg/kg/day Oncogenic: 10 mg/kg/day LOAEL: Chronic: 25 mg/kg/day Oncogenic: 25 mg/kg/day Chronic LOAEL: Cell hyperplasia, hypertrophy in the urinary bladder. Carcinogenic LOAEL: Benign submucosal mesenchymal tumours in the urinary bladder	Acceptable Deviations with respect to OECD TG 451: Hematology should have been performed for all animals instead of 10 used in this study	Anonymous 52, 1997
Telone® Soil fumigant: two-year **dietary** chronic toxicity/oncogenicity study in **B6C3F1** Mice. (microencapsulated) 0, 2.5, 25 and 50 mg/kg/day OECD TG 453 GLP Purity 95.8% 50.7% cis 45.1% trans	NOAEL: Chronic 2.5 mg/kg/day Oncogenic NOAEL 50 mg/kg/bw/day LOAEL: Chronic 25 mg/kg/day Chronic LOAEL: Depression of in-life bw /bw gain and food consumption. Not carcinogenic	This study is acceptable with some reservations. Deviations with respect to OECD Guideline No. 453: the group sacrificed at 12 months should have contained 20 animals/sex/group instead of the 10 used in this study. Hematology at three and six months should have been performed and 20 animals/sex/group should have been examined instead of 10. Urinalysis and clinical biochemistry were not conducted.	Anonymous 57, 1995

National Toxicology Program (NTP). (1985) Toxicology and carcinogenesis studies of Telone II® (technical-grade 1,3-dichloropropene containing 1% epichlorohydrin as astabilizer) in F344/N rats and B6C3F1 mice (gavage studies). U.S. Department of Health and Human Services Technical Report Series No. 269 B6C3F1 mice - 0, 50 and 100 mg/kg OECD Guideline – TG 451 GLP - yes	On the basis of urinary bladder and lung neoplasms in mice, the LOAEL for cancer in the study is 21.4 mg/kg (50 mg/kg/day × 3 days/7 days) and there is no NOAEL for mice	This study had major deficiencies with respect to OECD TG 451. Dosing three times in a week, the male mouse study was inadequate for investigation due to the greatly reduced survival in the vehicle control group . The current batches do not contain epichlorhydrin as stabilizer, thus the study results do not provided useful information for the current evaluation of test substance.	In: TOXICOLOGICAL REVIEW OF 1,3-DICHLOROPROPENE (CAS No. 542-75-6) - In Support of Summary Information on the Integrated Risk Information System (IRIS), *May 2000* - EPA/635/R-00/001 Draft Assessment Report, Spain, 2018

There are no rodent or non-rodent studies available for 1,3-D (mixture of isomers), cis-1,3-dichloropropene or trans-1,3- dichloropropene via the dermal route,

Table 105: Summary of animal studies on repeated inhalation dose toxicity study

Method	Results	Remarks	Reference
Rat studies			
Cis-1,3-Dichloropropene: 2-week vapor inhalation toxicity study in Fischer 344 rats Non-guideline study Groups of 15 male and 15 female rats were exposed to 0, 0.05, 0.27 or 0.68 mg/L/6h/day (0, 10, 60 and 150 ppm) vapors of the test material for 6 hours/day, 7 days/week for 9 exposures. Whole-body exposures occurred under dynamic airflow conditions. Cis-1,3-Dichloropropene 95.6%; Lot No. 2047. GLP Yes	NOAEL: 0.27 mg/L/6h/day (60 ppm), male/female. LOAEL: 0.68 mg/L/6h/day (150 ppm), male/female - based on changes in body weight, non-protein sulfhydryl levels at 1-hour in liver, kidney and lung and histopathological changes of moderate severity in the respiratory and olfactory mucosa in the nasal cavity in male and female rats exposed to 0.68 mg/L/6h/day (150 ppm)	Male and female rats exposed to 0.68 mg/L/6h/day (150 ppm) cis-DCP lost weight during the course of the two-week study. Body weights of male and female rats exposed to 0.27 mg/L/6h/day (60 ppm) were slightly decreased (3%) from control values at the end of the study. There were apparent exposure concentration-related decreases in liver, kidney and lung NPSH levels for male rats exposed to 0.68 mg/L/6h/day (150 ppm) cis-PCP, as well as a decrease in liver NPSH for males in the 0.27 mg/L/6h/day (60 ppm) exposure group when measured one hour after exposure. However, the NPSH values in all of these tissues were higher than control values by 18 hours post-exposure, and there were no associated gross or histopathologic	Anonymous 48 (1990)

		changes in liver, kidneys or lungs. The NPSH measurements for females were unremarkable. Histopathologic examination revealed changes of moderate severity in the respiratory and olfactory mucosa in the nasal cavity of males and female rats exposed to 0.68 mg/L/6h/day (150 ppm) cis-DCP; there were no exposure-related histopathologic changes in animals in the two lower exposure groups. Thus the no-adverse-effect-level (NOAEL) was 0.27 mg/L/6h/day (60 ppm).	
Cis-1,3-Dichloropropene: 13 week vapor inhalation toxicity study in Fischer 344 rats OECD 413 Groups of 10 male and 10 female rats were exposed to 0, 0.05, 0.14 or 0.41 mg/L/6h/day (0, 10, 30 and 90 ppm) of the test material for 6 hours/day, 5 days/week for 13 weeks. Whole-body exposures occurred under dynamic airflow conditions. Minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of the relevant results. Cis 1,3-Dichloropropene (cis-DCP); 94.3% cis; 1.5% trans, 0.2% 1,2-D (Shell analysis); 95.6% cis 1,3-D (additional analysis), Lot No. 2047. GLP Yes	NOEL: 0.14 mg/L/6h/day (30 ppm), male/female – based on decreased bodyweight of males and hyperplasia of the nasal and respiratory epithelia of males and females exposed to 0.41 mg/L/6h/day (90 ppm) LOEL: 0.41 mg/L/6h/day (90 ppm) (male/female) - based on decreased body weight of males and hyperplasia of the nasal and respiratory epithelia of males and females	Several exposure-related effects were observed in rats exposed to 0.41 mg/L/6h/day (90 ppm). Body weights of male rats were significantly decreased from control values throughout the 13-week exposure period (decreased 8% from control values at the end of the study); body weights of female rats were significantly decreased for the first six weeks (6%) but were comparable thereafter (decreased 2% from control values at the end of the study). As a result of the decreased body weight in male rats, several relative organ weights (kidneys, lungs, liver, and testes) were significantly elevated from control values. The relative liver weight of female rats was also increased from control values. These organ weight changes in male and female rats were unaccompanied by gross or histopathologic exposure-related changes and thus were	Anonymous 49 (1991)

		attributed to the body weight differences. Exposure-related microscopic changes occurred only in the nasal cavities of male and female rats exposed to 0.41 mg/L/6h/day (90 ppm) cis-DCP and consisted of slight multifocal bilateral degeneration of olfactory epithelium and slight bilateral multifocal hyperplasia of respiratory epithelium. There were no exposure-related effects noted in rats exposed to 0.05 or 0.14 mg/L/6h/day (10 or 30 ppm) cis-DCP. Thus, the no-observed-effect-level (NOEL) was 0.14 mg/L/6h/day (30 ppm) cis-DCP	
14-Day range-finding inhalation toxicity with Trans-D in rats OECD 412 The toxicity of Trans1,3-Dichloropropene (Trans-D) was studied in a 14-day range-finding study in Wistar rats, in order to establish the concentration levels of Trans-D to be tested during a subsequent sub-chronic (90-day) inhalation toxicity study also in Wistar rats. Four groups of 5 male and 5 female rats each were exposed to target concentrations of 0, 0.14, 0.45 or 1.36 mg/L/6h/day (0, 30, 100 or 300 ppm) for 6 hours a day, 5 days a week for 14 days in nose-only inhalation units. General condition, behaviour, body and organ weights and gross observations were used as criteria to disclose possible harmful effects. Trans1,3-Dichloropropene (Trans-D), Batch No. A4308 05008, Purity 96.7% GLP – Yes	Based on the results of the study, it was concluded that exposure for 14 days to 0.14, 0.45 or 1.36 mg/L/6h/day (30, 100 or 300 ppm) Trans-D was associated with a concentration-related decrease in body weight gain in all male exposure groups and in females of the mid and high concentration groups and, besides some other changes in relative organ weights, in a concentration-related increase in relative lung weight of males of the mid and high concentration group and of females of the high concentration group.	Supplemental — Based on these results, suggested levels for the 90-day inhalation toxicity study, therefore, were 0, 0.14, 0.45 or 1.36 mg/L/6h/day (0, 30, 100 or 300 ppm).	Anonymous 50 (1999)
Sub-chronic (90-day) inhalation toxicity study with Trans-1,3-Dichloropropene (Trans-D) in rats OECD 413 Four groups of 10 male and 10 female rats each were exposed to target concentrations of 0, 0.05, 0.14 or 0.41 mg/L/6h/day (0, 10, 30 or 90 ppm) for 6 hours a day, 5 days a week, for 13 weeks in a nose-only unit. Four additional group of 5 male and 5 female animals each, were similarly	Based on the observations noted in the study, the low concentration level, i.e. 0.04 mg/L/6h/day (8.9 ppm) , was considered a No-Observed-Adverse-Effect Level (NOAEL).	Acceptable study conforming to OECD guideline 413 and conducted in accordance with the Principles of GLP	Anonymous 51 (1999)

exposed during a period of 4 weeks. Clinical observations, growth, food consumption, food conversion efficiency, ophthalmoscopy, haematology, clinical chemistry, urinalysis, organ weights, gross examination at autopsy and microscopic examination of selected organs and tissues were used as criteria to identify possible harmful effects. Trans1,3-Dichloropropene (Trans-D), Batch No. A4308 05008, Purity 96.7% GLP - Yes			
Telone II soil fumigant: 13-week inhalation study in rats and mice. 0.0, 0.04, 0.13, 0.40, 0.67 mg/L/6hr/day (0, 10, 30, 90 or 150 ppm) Purity 90.9% 48.6% cis 42.3% trans Contains 1.2% epichlorohydrin as stabilizer Fischer 344 albino rats OECD TG 413 GLP	NOAEC: 0.04 mg/L (10 ppm) LOAEC: 0.13 mg/L (30 ppm) Significantly decreases in bw at ≥ 90 ppm, biologically relevant (18-20%) at 150 ppm Degeneration of the olfactory epithelium at 150 ppm (m/f) and/or hyperplasia of the nasal respiratory epithelium at ≥ 30 ppm (m) (f): Hypoplasia of the uterine tissue at 150 ppm. Uterine glands were not as numerous or as well developed as those of control.	**Not acceptable.** The batch used in this study does not comply with specification of the current batches, as it **contains epichlorohydrin** as a stabilizer thus the study results does not provide useful information for the current evaluation of test substance.	Anonymous, 73, (1984)
TELONE II SOIL FUMIGANT: **2-YEAR INHALATION** CHRONIC TOXICITY-ONCOGENICITY STUDY IN RATS **-- INTERIM REPORT:** 6- AND 12-MONTH INTERIM SACRIFICE OF RATS 0, 0.02, 0.09 or 0.27 mg/L/6h/day (0, 5, 20 or 60 ppm) Purity 92.1% 49.5% cis 42.6% trans Fischer 344 albino rats OECD TG 453 GLP	NOAEL: Chronic: 0.09 mg/L/6h/day (20 ppm) LOAEL: Chronic: 0.27 mg/L/6h/day (60 ppm) Slight depression of in-life body weights of both sexes.	This study is acceptable with some reservations. Deviations with respect to OECD TG 453: the groups sacrificed at 6 and 12 months (interim report) should have contained 20 animals/sex/group instead of the 10 used in this study. Haematology and urinalysis at three months should have been performed. Ophthalmological examinations not conducted. Food consumption not conducted	Anonymous 147, 1985 *Interim report (6 & 12 month exposure) to Anonymous 58, 1987 (2 year exposure)*
TELONE* II SOIL FUMIGANT: **2-YEAR INHALATION** CHRONIC TOXICITY-ONCOGENICITY STUDY IN RATS 0, 0.02, 0.09 or 0.27 mg/L/6h/day (0, 5, 20 and 60 ppm) Purity 92.1%	NOAEC: Chronic: 0.09 mg/L/6h/day (20 ppm) LOAEC: Chronic: 0.27 mg/L/6h/day (60 ppm)	This study is acceptable with some reservations. Deviations with respect to OECD TG 453: Haematology and urinalysis at three months should have been performed.	Anonymous 58, 1987 *Final report (2 year exposure). Interim report above (Anonymous 55, 1985) contains 6 & 12 month exposures*

49.5% cis 42.6% trans Fischer 344 albino rats OECD TG 453 GLP	Depression of body weights and Microscopic changes of nasal epithelium. (Decreased thickness olfactory epithelium, Erosions of olfactory epithelium, Submucosal Fibrosis) **Not carcinogenic.**	Ophthalmological examinations not conducted. Food consumption not conducted. Note: results from the animals sacrificed after 6 and 12 months of exposure level have been reported previously (Anonymous 55, 1985)	
Mouse studies			
Telone II soil fumigant: 13-week inhalation study in rats and mice. 0.0, 0.04, 0.13, 0.40, 0.67 mg/L/6hr/day (0, 10, 30, 90 or 150 ppm) Purity 90.9% 48.6% cis 42.3% trans Contains 1.2% epichlorohydrin as stabilizer B6C3F1 mice OECD TG 413 GLP	NOAEC: 0.13 mg/L (30 ppm) LOAEC: 0.40 mg/L (90 ppm) Significantly decreases in bw at ≥ 90 ppm, biologically relevant (10-12%) at 150 ppm. (m/f) Significantly decreases in absolute (14-24%) and relative (12-14%) liver weights at ≥ 90 ppm (m) and ≥ 150 ppm (f) Slight degeneration of olfactory epithelium. Slight hyperplasia of nasal respiratory epithelium with focal areas of metaplasia at ≥ 90 ppm (m/f) Hyperplasia of urinary bladder transitional epithelium in females at ≥ 90 ppm	**Not acceptable.** The batch used in this study does not comply with specification of the current batches, as it **contains epichlorohydrin** as a stabilizer thus the study results does not provide useful information for the current evaluation of test substance.	Anonymous, 73, (1984)
TELONE* II SOIL FUMIGANT: 2-YEAR **INHALATION** CHRONIC TOXICITYONCOGENICITY STUDY IN MICE -- INTERIM REPORT: 6- AND 12- MONTH EXPOSURES 0, 0.02, 0.09 or 0.27 mg/L/6h/day (0, 5, 20 and 60 ppm) B6C3F1 mice OECD TG 453 GLP Purity 92.1% 49.5% cis 42.6% trans	NOAEC: Chronic: 0.02 mg/L/6h/day (5 ppm) LOAEC: Chronic: 0.09 mg/L/6h/day (20 ppm) Hyperplasia and hypertrophy of the respiratory epithelium of the nasal turbinates	This study is acceptable with some reservations. Deviations with respect OECD TG 453: there were only 10 (as opposed to 20) mice/sex/dose in the satellite groups for 6 and 12-month sacrifices; food consumption not conducted; ophthalmology not conducted; haematological examination was not conducted at 3 months; urinalysis was not conducted. Note: This study was conducted as a combined study; however, the reporting of satellite group data was done separately from the main/onco group.	Anonymous 55, 1985 *Interim report (6 & 12 month exposure) to Anonymous 56, 1987 (2 year exposure)*

Telone Soil Fumigant **2-year inhalation** chronic toxicity/oncogenicity study in mice 0, 0.02, 0.09 or 0.27 mg/L/6h/day (0, 5, 20 and 60 ppm) B6C3F1 mice OECD TG 453 GLP Purity 92.1% 49.5% cis 42.6% trans	NOAEL: Chronic: 0.02 mg/L/6h/day (5 ppm) Oncogenic: 0.09 mg/L/6h/day (20 ppm) LOAEL: Chronic: 0.09 mg/L/6h/day (20 ppm) Oncogenic: 0.27 mg/L/6h/day (60 ppm) **Chronic LOAEL**: Lesions of the urinary bladder and nasal mucosa. Hyperplasia of the epithelial of the non-glandular portion of the stomach. Decreased body weight and modified organ weight **Carcinogenic LOAEL: Increase incidence of benign** lung **tumours.**	This study is acceptable with some reservations. Deviations with respect OECD 453: Only haematology and clinical chemistry of the 24-month exposure group were present in this report. Urinalyses was not conducted. Food consumption was not recorded. In period of holidays the treatment was not given. In some haematological parameters statistical comparison of means was not conducted.	Anonymous 56, 1987 *Final report (2 year exposure). Interim report above (Anonymous 55, 1985) contains 6 & 12 mth exposures*

10.12.1 Comparison with the CLP criteria

According to Regulation (EC) No. 1272/2008 (the CLP Regulation) and ECHA Guidance on the Application of the CLP Criteria, Version 5.0, July 2017, the criteria for classifying substances for specific target organ toxicity-repeated exposure are as follows and refer to the outcome of 90 day studies:

Category 1: Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. 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. Guidance dose/concentration values for 90-day studies are provided below, to be used as part of a weight-of- evidence evaluation:

Guidance values for 90-day studies to assist in Category 1 classification

Route of exposure	Units	Guidance values (dose/concentration)
Oral (rat)	mg/kg body weight/day	C ≤ 10
Dermal (rat or rabbit)	mg/kg body weight/day	C ≤ 20
Inhalation (rat) gas	ppmV/6h/day	C ≤ 50
Inhalation (rat) vapour	mg/litre/6h/day	C ≤ 0,2
Inhalation (rat) dust/mist/fume	mg/litre/6h/day	C ≤ 0,02

Category 2: Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values for 90-day studies are provided below in order to help in classification:

Guidance values for 90-day studies to assist in Category 2 classification

Route of exposure	Units	Guidance values (dose/concentration)
Oral (rat)	mg/kg body weight/day	10 < C ≤ 100

Dermal (rat or rabbit)	mg/kg body weight/day	20 < C ≤ 200
Inhalation (rat) gas	ppmV/6h/day	50 < C ≤ 250
Inhalation (rat) vapour	mg/litre/6h/day	0,2 < C ≤ 1,0
Inhalation (rat) dust/mist/fume	mg/litre/6h/day	0,02 < C ≤ 0,2

Repeated oral dose toxicity studies (rats)

The first study, the chronic toxicity and oncogenicity study, Anonymous 53 (1998), is not judged to be a reliable assessment of repeat dose toxicity. It is acknowledged that technical difficulties with the gavage dosing methodology contributed to a low and variable survival rate (as low as 22% after two years) in all groups including controls. The chronic LOAEL was set at 10 mg/kg/day, and microscopic morphologic abnormalities consisting of a dose-related increased incidence of squamous cell hyperplasia and hyperkeratosis were observed in the forestomach of males and females at 10 and 25 mg/kg bw/day dosages at the 12-month interim sacrifice (see section 10.9.1) which fall just within the Annex I: 3.9.2.9.7 range extrapolated to 1-year exposure (>2.5 to ≤ 25 mg/kg/day) for oral (rat) Cat. 2 classification, although no HCD were provided.

Histopathologic Observations in rats at 12-Months (Stomach)

Sex	**Males**				**Females**			
Dosage (mg/kg bw/day)	**0**	**2**	**10**	**25**	**0**	**2**	**10**	**25**
12-Month Sacrifice Animals								
Number of Mice Examined	9	10	9	9	7	10	10	9
Fore-stomach – squamous cell hyperplasia								
Minimal	2	0	3	7	0	1	2	3
Slight	1	0	2	0	1	0	2	5
Total	3	0	5	7	1	1	4	8
Fore-stomach – hyperkeratosis								
Minimal	3	0	4	6	1	1	3	3
Slight	0	0	1	2	0	0	1	5
Total	3	0	5	8	1	1	4	8
Premature decedents up to end of 12 months								
Number of Mice Examined	14	11	14	11	7	6	7	2
Fore-stomach – squamous cell hyperplasia								
Minimal	0	0	1	2	1	1	0	1
Slight	0	1	0	4	0	1	1	0
Moderate	0	1	0	0	1	0	0	0
Total	0	2	1	6	2	2	1	1
Fore-stomach – hyperkeratosis								
Minimal	0	1	2	5	1	1	0	1
Slight	0	0	1	1	1	1	2	0
Moderate	0	1	0	0	0	0	0	0
Total	0	2	3	6	2	2	2	1

A second study, a 90-day toxicity study, Anonymous 146 (1993), was considered acceptable and relevant. Hyperkeratosis and basal cell hyperplasia of the non-glandular mucosa of the stomach was observed in male and female animals with a NOAEL of 5 mg/kg/day and a LOAEL of 15 mg/kg/day. This 90-day LOAEL of 15 mg/kg/day and effects up to 100 mg/kg bw/day fall within the Annex I: 3.9.2.9.7 range (10 to ≤100 mg/kg/day) for oral (rat) and stomach hyperplasia, which is considered to be the type of regenerative damage covered by the STOT-RE criteria, was a reported finding from 15.0 mg/kg/day. **Therefore, an oral (rat) STOT-RE Cat. 2 classification is applicable to this study.**

Histopathologic Observations (Stomach and Mesenteric Tissue)

Sex	**Males**					**Females**				
Dose in mg/kg/day	**0**	**5**	**15**	**50**	**100**	**0**	**5**	**15**	**50**	**100**
Number of Rats Examined	10	10	10	10	10	10	10	10	10	10
Stomach (# of tissues examined)	10	10	10	10	10	10	10	10	10	10
Within normal limits	7	8	3	0	0	9	10	5	0	0

Sex	Males					Females				
Dose in mg/kg/day	**0**	**5**	**15**	**50**	**100**	**0**	**5**	**15**	**50**	**100**
Cystic dilatation, glandular mucosa, focal: - very slight	0	0	0	0	0	0	0	2	1	0
Hyperkeratosis, nonglandular mucosa: - slight	0	0	1	3	3	0	0	0	3	5
Mineralization, glandular mucosa, multifocal: - very slight	3	2	4	2	5	0	0	0	0	0
Hyperplasia - basal cell, nonglandular mucosa: - very slight	0	0	4	9	6	1	0	3	10	6
Hyperplasia - basal cell, nonglandular mucosa: - slight	0	0	0	1	4	0	0	0	0	4
Mesenteric Tissues (# of tissues examined)	9	1	0	0	10	10	10	10	10	10
Missing:	1	0	--	--	0	0	0	0	0	0
Within normal limits:9	9	0	--	--	9	10	10	10	9	1
Atrophy, adipose tissue:	0	0	--	--	0	0	0	0	1	9
Mineralization, blood vessels, multifocal: - slight	0	0	--	--	1	0	0	0	0	0
Strangulated or necrotic fat, focal:	0	1	--	--	0	0	0	0	0	0

--: not calculated when weight gain was negative

A third study, two-year chronic toxicity /oncogenicity study, Anonymous 54 (1995), was reported to have an acceptable mortality rate and the report mentions the use of HCD to contextualize the findings on the study. With the exceptions of the deficiencies listed above, the study was deemed acceptable, with reservations. The chronic LOAEL was set at 12.5 mg/kg/day which falls within the Annex I: 3.9.2.9.7 range (2.5 to ≤25 mg/kg/day; adjusted for 1 year chronic exposure) based on stomach hyperplasia, which is considered to be the type of regenerative damage covered by the STOT-RE criteria, and was a reported finding at 12.5 mg/kg/day. The CLH report (page 102) theorizes that epichlorohydrin, a Telone II stabilizer, might be in part responsible for hyperplasia and squamous cell papilloma/carcinoma findings in the rat forestomach (drinking water study, Konisihi et al, 1980) however this later Telone II without epichlorohydrin feeding study (Anonymous 54, 1995) did report forestomach hyperplasia in rats. **Therefore, an oral (rat) STOT-RE Cat. 2 classification is applicable to this study.**

Rat 12-Month Histopathology—Stomach (study Anon, 54 (1995)

Sex	Males				Females			
Dose in mg/kg/day	**0**	**2.5**	**12.5**	**25**	**0**	**2.5**	**12.5**	**25**
Number of Rats Examined	10	10	10	10	10	10	10	10
Stomach (# of Tissues Examined)	10	10	10	10	10	10	10	10
Within normal limits:	6	6	2	0	8	7	7	1
Aggregate(s) of mononuclear (predominately lymphoid) cells, glandular mucosa, multifocal: -very slight	2	2	3	2	2	2	0	1
Aggregate(s) of mononuclear (predominately lymphoid) cells, glandular mucosa, multifocal: -slight	0	0	0	0	0	1	0	0
Aggregate(s) of mononuclear (predominately lymphoid) cells, glandular mucosa, multifocal: -any severity	2	2	3	2	2	3	0	1
Mineralization, muscularis, focal:	0	0	0	1	0	0	0	0
Mineralization, blood vessels, focal:	1	2	1	2	0	0	0	0
Mineralization, blood vessels, multifocal:	1	0	1	0	0	0	0	0
Mineralization, blood vessels, focal or multifocal:	2	2	2	2	0	0	0	0
Hyperplasia – basal cell, non-glandular mucosa: -very slight	0	1	5	6	0	0	2	7
Hyperplasia – basal cell, non-glandular mucosa: -slight	0	0	2	4	0	0	1	2
Hyperplasia – basal cell, non-glandular mucosa: -any severity	0	1	7	10	0	0	3	9

The National Toxicology Program (NTP) (1985) study on Toxicology and carcinogenesis studies of Telone II® (technical-grade 1,3-dichloropropene containing 1% epichlorohydrin as a stabilizer) in F344/N rats and B6C3F1 mice (gavage studies). U.S. Department of Health and Human Services Technical Report Series No. 269, was disregarded for STOT-RE Cat. 2 classification purposes due to major deficiencies. For example, the presence of a genotoxic stabilizer in the dose preparations and the animals were dosed three times per week. The NOAEL (for rats) was set at 25 mg/kg/day and the LOAEL was set at 50 mg/kg/day based on forestomach and liver neoplasms in the rat. The effects are shown below.

Treatment-related Nonneoplastic and Neoplastic Lesions

		Males			**Females**		
Concentration (mg/kg/day)		**0**	**50**	**100**	**0**	**50**	**100**
Nonneoplastic:							
	Forestomach, basal cell hyperplasia	NV	0/50	4/50	1/50	1/50	21/50
	Kidney, hydronephrosis	NV	0/50	0/50	0/50	2/50	14/50
	Urinary bladder, epithelial hyperplasia	NV	9/50	18/50	2/50	15/50	19/48
Neoplastic:							
	Forestomach papilloma	NV	2/52	3/50	0/50	1/50	2/50
	Forestomach carcinoma	NV	0/52	4/52	0/50	0/50	2/50
	Urinary bladder carcinoma	NV	0/50	2/50	0/50	8/50*	21/48*
	Alveolar/bronchiolar adenoma	NV	11/50	9/50	0/50	3/50	8/50*$

NV—invalid study due to high mortality in control male mice.
*Statistically identified as difference from control.
$Linear trend statistically identified.

The 90-day rat inhalation study by Anonymous 73 (1984) is considered unacceptable because it tested a batch if 1,3-D known to contain epichlorohydrin, added as a stabilizing agent. Epichlorohydrin is an in vitro and in vivo genotoxicant, and will confound the test results. Therefore, the data from this study is not considered as acceptable for the assessment of the repeat dose toxcity of 1,3-D and are not further discussed.

Repeated oral dose toxicity studies (mice)

In an 18-month oncogenicity study by Anonymous 52 (1997), the mortality rate was reported to be acceptable; however, the report does not mention the use of HCD to put into context the findings of the study. With the exceptions of the deficiency listed above, the study was deemed acceptable. The chronic LOAEL for hypertrophy and hyperplasia of the urinary bladder was set at 25 mg/kg/day (see the incidences for non-neoplastic findings in Table 71). As this value falls outside of the Annex I: 3.9.2.9.7 range (10 to ≤100 mg/kg/day, which can be adjusted to 1.67-16.7 mg/kg/day for a 18-month study), this study can be disregarded for STOT-RE Cat. 2 classification purposes.

The findings on the study by Anonymous 57 (1995) were limited to slight reductions in bodyweight, albeit dose related. Food consumption and organ weights reflected the bodyweights. There were no treatment-related histopathologic effects at the 12-month or 24-month sacrifices in males administered 2.5 or 25 mg/kg /day TELONE II or in females administered 2.5, 25, 50 mg/kg /day TELONE II. A treatment-related histopathologic effect was observed in the liver of male mice administered 50 mg/kg /day TELONE II (see section 10.9.1). The liver effect consisted of diffuse, slightly decreased size of hepatocytes, which was attributed to a decrease in hepatocellular cytoplasmic area as compared to hepatocytes from control group rats. This change was consistent with a decrease in hepatocellular cytoplasmic glycogen, and corresponded to a statistically identified decrease in absolute liver weights at this dose level. Decreased size of hepatocytes was only noted in rats from the 12-month sacrifice, According to Annex I: 3.9.2.8, effects considered not to support classification for STOT-RE are small changes in bodyweight gain, food consumption or changes in organ weights with no evidence of organ dysfunction. Although the chronic LOAEL was set at 25 mg/kg/day, which falls within the Annex I: 3.9.2.9.7 range (10 to ≤100 mg/kg/day which can be adjusted to 2.5-25 mg/kg/day for a 12-month exposure) for oral (rat), these effects are considered not to support STOT-RE Cat. 2 classification.

The National Toxicology Program (NTP) (1985) study on Toxicology and carcinogenesis studies of Telone II® (technical-grade 1,3-dichloropropene containing 1% epichlorohydrin as a stabilizer) in F344/N rats and B6C3F1 mice (gavage studies). U.S. Department of Health and Human Services Technical Report Series No.

269, was disregarded for STOT-RE Cat. 2 classification purposes due to major deficiencies. The effects are shown below.

Treatment-related Nonneoplastic and Neoplastic Lesions

		Males (mg/kg/day)			Females (mg/kg/day)		
Gavage		(0)	50	100	0	50	100
Nonneoplastic:							
	Forestomach, basal cell hyperplasia	NV	0/50	4/50	1/50	1/50	21/50
	Kidney, hydronephrosis	NV	0/50	0/50	0/50	2/50	14/50
	Urinary bladder, epithelial hyperplasia	NV	9/50	18/50	2/50	15/50	19/48
Neoplastic:							
	Forestomach papilloma	NV	2/52	3/50	0/50	1/50	2/50
	Forestomach carcinoma	NV	0/52	4/52	0/50	0/50	2/50
	Urinary bladder carcinoma	NV	0/50	2/50	0/50	8/50*	21/48*
	Alveolar/bronchiolar adenoma	NV	11/50	9/50	0/50	3/50	8/50*$

NV--invalid study due to high mortality in control male mice.
*Statistically identified as difference from control.
$Linear trend statistically identified.

Repeated inhalation dose toxicity studies (rats)

In the first study (Anonymous 48, 1990), the purpose was to determine the potential inhalation toxicity of cis-1,3-dichloropropene (cis-DCP) vapor in rats. Groups of 15 male and 15 female rats were exposed to 0, 0.04, 0.27 and 0.68 mg/L/6h/day (0, 10, 60 and 150 ppm vapors) of the test material for 6 hours/day, 7 days/week for 9 exposures. Whole-body exposures occurred under dynamic airflow conditions. Animals were observed daily and weighed at selected intervals. On the day after the last exposure, 5 male and 5 female rats/exposure level were necropsied. Major organs were weighed and selected tissues were evaluated histopathologically. Groups of five animals/sex/exposure level were used to determine nonprotein sulfhydryl (NPSH) content in the liver, kidney and lung at 1 and at 18 hours, respectively, following the last exposure.

Male and female rats exposed to 0.68 mg/L/6h/day (150 ppm) cis-DCP lost weight during the course of the two-week study. Body weights of male and female rats exposed to 0.27 mg/L/6h/day (60 ppm) were slightly decreased (3%) from control values at the end of the study. There were apparent exposure concentration-related decreases in liver, kidney and lung NPSH levels for male rats exposed to 0.68 mg/L/6h/day (150 ppm) cis-PCP, as well as a decrease in liver NPSH for males in the 0.27 mg/L/6h/day (60 ppm) exposure group when measured one hour after exposure. However, the NPSH values in all of these tissues were higher than control values by 18 hours post-exposure, and there were no associated gross or histopathologic changes in liver, kidneys or lungs. The NPSH measurements for females were unremarkable. Histopathologic examination revealed changes of moderate severity in the respiratory and olfactory mucosa in the nasal cavity comprising diffuse bilateral degeneration of olfactory epithelium, grade moderate in 5/5 males and 5/5 females at 0.68 mg/L/6h/day, and bilateral multifocal hyperplasia of respiratory epithelium grade moderate in 5/5 males in 5/5 females at 0.68 mg/L; all animals in control group and at 0.05 and 0.27 mg/L/6h/day had no adverse findings in the nasal tissues. Based on these findings the LOAEL was set at 0.68 mg/kg/6h/day (150 ppm) and the NOAEL was set at 0.27 mg/L/6h/day (60 ppm). .

The second study (Anonymous 49, 1991) was a GLP-compliant repeated dose inhalation study conducted in accordance with OECD Guideline 413. The purpose of this study was to determine the potential inhalation toxicity of cis-1,3-dichloropropene (cis-DCP) vapor in rats. Groups of 10 male and 10 female rats were exposed to 0, 0.04, 0.14 and 0.41 mg/L/6h/day (0, 10, 30 and 90 ppm) of the test material for 6 hours/day, 5 days/week for 13 weeks. Whole-body exposures occurred under dynamic airflow conditions. Animals were observed daily and weighed at weekly intervals. All animals were necropsied on the day after the last exposure. Major organs were weighed, and tissues were evaluated histopathologically.

Several exposure-related effects were observed in rats exposed to 0.41 mg/L/6h/day (90 ppm). There were no adverse findings at the mid and low dose levels (0.05 and 0.14 mg/L/6h/day). Body weights of male rats were significantly decreased from control values throughout the 13-week exposure period (decreased 8% from control values at the end of the study); body weights of female rats were significantly decreased for the first six weeks (6%) but were comparable thereafter (decreased 2% from control values at the end of the study). As

a result of the decreased body weight in male rats, several relative organ weights (kidneys, lungs, liver, and testes) were significantly elevated from control values. The relative liver weight of female rats was also increased from control values. These organ weight changes in male and female rats were unaccompanied by gross or histopathologic exposure-related changes and thus were attributed to the body weight differences. Exposure-related microscopic changes occurred only in the nasal cavities of male and female rats exposed to 0.41 mg/L/6h/day (90 ppm) cis-DCP and consisted of slight multifocal bilateral degeneration of olfactory epithelium and slight bilateral multifocal hyperplasia of respiratory epithelium. There were no exposure-related effects noted in rats exposed to 0.04 or 0.14 mg/L/6h/day (10 or 30 ppm) cis-DCP. Thus, the no-observed-effect-level (NOEL) was 0.14 mg/L/6h/day (30 ppm) cis-DCP. However, the changes in organ weights can be attributed to decreased body weight changes and there is also no associated changes in histopathology. The only treatment related change is the microscopic changes in the nasal cavity of rats (both sexes) exposed to the highest dose of 0.41 mg/L/6h/day (90 ppm).

In the third study (Anonymous 50, 1999), the toxicity of Trans1,3-Dichloropropene (Trans-D) was studied in a 14-day range-finding study in Wistar rats, in order to establish the concentration levels of Trans-D to be tested during a subsequent sub-chronic (90-day) inhalation toxicity study also in Wistar rats. Four groups of 5 male and 5 female rats each were exposed via nose-only administration to target concentrations of 0, 0.14, 0.45 or 1.36 mg/L/6h/day (0, 30, 100 or 300 ppm) for 6 hours a day, 5 days a week for 14 days. General condition, behaviour, body and organ weights and gross observations were used as criteria to disclose possible harmful effects.

Mean actual concentrations were close to the target concentrations and were 0.12 mg/L/6h/day (27.3 ± 0.8 ppm), 0.42 mg/L/6h/day (91.7 ± 2.1 ppm) and 1.21 mg/L/6h/day (267.8 ± 2.3 ppm) for the low, mid and high concentration, respectively.

During exposure, changes in breathing pattern were observed such as superficial breathing at a visually reduced frequency in animals of the mid and high concentration group on the first exposure day, and a slightly reduced breathing frequency in animals of the mid and high concentration on most of the other exposure days. No abnormalities were observed after exposure. Individual observations in the mornings revealed sparsely haired skin in 3 out of 5 males and all 5 females of the mid and high concentration group. All animals survived until scheduled necropsy. A concentration-related decrease in body weight gain was observed in male rats. In females, body weight loss was observed in females of the high concentration group during the entire period. Females of the mid concentration group showed a significantly decreased body weight on day 14. No statistically significant changes in body weight were observed in females of the low concentration group. A concentration-related decrease in food intake and food conversion efficiency was observed in all exposure groups when compared to controls. A concentration-related increase in relative lung weight was observed in males of the mid and high concentration group. Females of the high concentration group also showed a significantly increased relative lung weight. Statistically significant increases were further observed in relative weights of the adrenals (males and females of the high concentration group), kidneys (males and females of the high concentration group), brain (males and females of the high concentration group and males of the mid concentration group), and head (males of the mid concentration group). Gross examination at the end of the treatment period did not reveal treatment-related changes other than sparsely haired areas of the skin, observed in 3 out of 5 males of the high concentration group and all 5 females of the mid and high concentration group.

Based on the results of the study, it was concluded that exposure for 14 days to 0.14, 0.45 or 1.36 mg/L/6h/day (30, 100 or 300 ppm) Trans-D was associated with a concentration-related decrease in body weight gain in all male exposure groups and in females of the mid and high concentration groups and, besides some other changes in relative organ weights, in a concentration-related increase in relative lung weight of males of the mid and high concentration group and of females of the high concentration group. Based on these results, suggested levels for the 90-day nose only inhalation toxicity study, therefore, were 0, 0.14, 0.45 or 1.36 mg/L/6h/day (0, 10, 30 and 90 ppm). In conclusion the only findings in this 14 day nose-only inhalation study were small changes in bodyweight gain, food consumption and organ weights with no evidence of organ dysfunction.

In the fourth study (Anonymous 51, 1999), the inhalation toxicity of Trans-1,3-Dichloropropene (Trans-D) was studied in a sub-chronic (90-day) study in Wistar rats. Four groups of 10 male and 10 female rats each were exposed via nose-only administration to target concentrations of 0, 0.04, 0.14 or 0.41 mg/L/6h/day (0, 10, 30 or 90 ppm) for 6 hours a day, 5 days a week, for 13 weeks in a nose-only unit. Four additional group of

5 male and 5 female animals each, were similarly exposed during a period of 4 weeks. Clinical observations, growth, food consumption, food conversion efficiency, ophthalmoscopy, haematology, clinical chemistry, urinalysis, organ weights, gross examination at autopsy and microscopic examination of selected organs and tissues were used as criteria to identify possible harmful effects.

Mean actual concentrations were close to the target concentrations and were 0.04 mg/L/6h/day (8.9 ± 0.1) ppm, 0.12 mg/L/6h/day (26.9 ± 0.5 ppm) and 0.37 mg/L/6h/day (80.7 ± 1.4 ppm) for the low, mid and high concentrations, respectively.

During exposure, no changes in breathing pattern or behaviour were observed. No abnormalities were observed after exposure. Individual daily observations prior to exposure revealed sparsely haired skin in females of the mid and high concentration group. A decrease in body weight gain was observed in male and female rats of the mid and high concentration groups. A decrease in food intake was seen in animals of the high concentration group. Food conversion efficiency was also decreased in males of this group.

In plasma obtained from animals of the high concentration 0.41 mg/L/6h/day (90 ppm) main study group, increases in alanine aminotransferase, triglyceride (females only) and in the albumin/globulin ration (males only) were found. An increase in urinary volume and a tendency towards a decreased urinary density were found in female animals of the high concentration main study group. Relative weight of the liver was increased in male and female animals of the high concentration main study group and in females of the high concentration interim kill group. Relative weight of the kidneys of females of the high concentration main study group was also increased. No treatment-related gross abnormalities at necropsy, nor histopathological changes at microscopic examination of organs and tissues were found. In the low concentration group (0.04 mg/L/6h/day≡8.9 ppm), no effects were seen that were considered of toxicological significance; therefore, this dose level was considered a No-Observed-Adverse-Effect Level (NOAEL). The only treatment related change was that seen in the plasma of rats (both sexes) exposed to the highest dose of 0.41 mg/L/6h/day (90 ppm). In conclusion the only findings in this 90-day nose-only inhalation study were small changes in bodyweight gain, food consumption andchanges in organ weights with no evidence of organ dysfunction.

Anonymous 147 (1985) was an interim study covering 6- and 12-month exposure periods and was supplementary to the later 2-year study (Anonymous 58, 1987). Four groups of 10 male and 10 female Fischer 344 rats each were exposed to 1,3-Dichloropropene (1:1 cis:trans) via whole body administration to target concentrations of 0, 0.02, 0.09, or 0.27 mg/L/6h/day (0, 5, 20 or 60 ppm) for 6 hours a day, 5 days a week, for 6 or 12 months. The report does not mention the use of HCD to put into context the findings of this interim study. The findings were limited to a slight depression of in-life bodyweights of rats of both sexes exposed to 0.27 mg/L/6h/day (60 ppm) which formed the basis of setting the chronic LOAEL at this dose level. There were no effects evident at 0.09 mg/L/6h/day (20 ppm) therefore establishing a NOAEL at this dose level. In conclusion the only findings were small changes in bodyweight gain with no evidence of organ dysfunction following inhalation exposure (rat) vapour for 6- and 12 months,

In Anonymous 58 (1987), Four groups of 50 male and 50 female Fischer 344 rats each were exposed to 1,3-Dichloropropene (1:1 cis:trans) via whole body administration to target concentrations of 0, 0.02, 0.09, or 0.27 mg/L/6h/day (0, 5, 20 or 60 ppm) for 6 hours a day, 5 days a week, for 2-years. The mortality rate in this 2-year study was reported to be acceptable and the report mentions the use of HCD to contextualize the findings on the study. With the exceptions of the deficiencies listed in Table 109 above, the study was deemed acceptable, with reservations. Based on the lack of findings at the 0.02 or 0.09 mg/L/6h/day (5 or 20 ppm) concentrations the chronic NOAEL was set at 0.09 mg/L/6h/day. The chronic LOAEL was set at 0.27 mg/L/6h/day (60 ppm or 60 mg/L) at which olfactory epithelial changes were reported. The only other finding was a small reduction in bodyweight gain, with no adverse effects in any other organs apart from site of contact effects in the nasal tissues.

Results

No clinical signs of toxicity were observed in exposed animals throughout the study.
There were no apparent increases in palpable masses due to exposure to TELONE II soil fumigant. No significant differences in survival were observed between any groups of either sex.

Percent of survival—2 year.

Doses (ppm)	0		5		20		60	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
	46%	60%	56%	52%	60%	76%	56%	72%

Mean body weights of both males and females exposed to 0.27 mg/L/6h/day (60 ppm) were statistically decreased from mean control values. For high exposure male rats, mean body weights were decreased approximately 5% from control male rats on test days 13- 425, but were similar to control rats throughout the remainder of the study. High exposure female rats had a similar mean body weight decrease of approximately 5% on test days 6-327 and were similar to control values throughout the remainder of the study.

Body Weights-2 Years

Conc. (mg/L/6h/day)	**0**		**0.02 (5 ppm)**		**0.09 (20 ppm)**		**0.27 (60 ppm)**	
Sex	**Male**	**Fem**	**Male**	**Fem**	**Male**	**Fem**	**Male**	**Fem**
Days on Test								
-1	150.0	108.3	151.8	107.9	153.3	107.6	148.8	107.4
6	179.4	126.1	180.3	125.5	182.7	126.6	172.4	121.0*
13	201.8	135.4	204.7	135.1	203.1	135.6	193.1*	129.0*
20	225.6	147.1	230.0	147.8	225.2	146.7	214.7*	139.1*
27	240.5	155.6	245.0	155.4	239.7	154.8	231.1*	147.9*
34	255.3	162.3	259.3	163.5	255.4	163.8	246.2*	155.5*
41	262.3	164.7	265.3	164.1	263.5	167.1	253.8*	158.9*
48	275.6	171.1	276.6	170.7	277.0	174.0	265.9*	163.7$
55	283.6	175.8	284.0	173.9	283.5	176.7	274.4*	168.6*
62	295.5	181.9	292.6	179.6	291.7	192.0	283.3*	174.1*
69	302.1	185.1	299.7	182.9	299.8	185.2	292.5*	177.8*
76	308.9	18.4	303.1	187.2	305.9	187.7	296.8*	181.1*
83	313.4	189.2	314.4	189.9	313.7	190.6	304.8*	185.4
90	321.4	193.6	322.6	194.2	316.6	191.8	307.6*	184.7*
117	344.1	201.6	342.4	202.0	334.9*	199.8	325.6*	193.1*
146	356.9	206.3	356.1	205.8	357.1	207.3	345.2*	200.5*
173	371.2	211.9	372.0	212.1	364.8	210.2	355.8*	204.6*
201	386.6	216.7	387.3	217.4	376.5*	215.0	369.4*	210.2*
229	401.7	222.3	400.6	221.4	387.1*	222.5	382.8*	214.7*
257	410.7	225.8	409.6	225.7	402.9	228.6	396.4*	221.4
285	421.6	231.3	418.4	231.8	405.2$	228.2	399.6$	224.7*
327	423.8	238.4	421.1	238.3	412.9*	236.4	408.0*	230.6*
341	420.7	236.0	419.3	238.4	413.0	236.0	406.5$	232.4
369	525.1	242.2	422.9	246.9	418.5	245.8	412.5$	240.8

Conc. (mg/L/6h/day)	**0**		**0.02 (5 ppm)**		**0.09 (20 ppm)**		**0.27 (60 ppm)**	
Sex	**Male**	**Fem**	**Male**	**Fem**	**Male**	**Fem**	**Male**	**Fem**
Days on Test								
397	528.9	248.5	425.3	252.3	417.8	250.1	410.6$	248.2
425	429.1	250.2	426.9	255.2	422.3	254.1	415.4$	251.8
453	432.7	259.2	434.2	262.3	430.8	262.9	422.6	259.9
481	430.7	266.5	432.8	267.3	432.6	270.2	423.3	265.6
509	430.3	269.8	434.0	271.5	428.0	272.4	424.0	267.2
537	434.0	274.9	436.5	271.5	429.2	280.5	424.6	272.0
565	431.9	274.9	436.3	269.7	430.1	286.2*	424.7	275.9
593	432.0	278.4	434.9	273.7	426.9	292.2	420.0	279.9
621	432.3	280.2	431.5	277.3	425.2	287.6	418.3	281.7
649	525.7	284.0	433.6	278.2	421.4	289.8	408.6	283.2
677	404.8	280.0	403.4	278.9	402.1	284.7	396.6	281.9
705	402.2	280.8	394.6	283.7	405.4	289.4	401.3	284.6
733	413.5	282.5	396.5	286.9	391.2	281.0	389.8	288.9

*Statistically different from control mean by Dunnett's test, alpha = 0.05.

$Statistically different from control mean by Wilcoxon's test, alpha = 0.05.

Haematology: there were no statistically identified differences in any haematology parameter examined between mean control values and values from exposed male and female rats.

Clinical chemistry: Female rats exposed to 0.27 mg/L/6h/day (60 ppm) had statistically identified decreases in mean total protein and albumin concentrations. Apart from these findings no other statistical differences between exposed and control male rats were observed.

Clinical Chemistries - (Total Protein, Albumin)

Concentration (mg/L/6h/day)	**Conc. (ppm)**	**TP g/dL**		**ALB g/dL**	
		Male	Fem	Male	Fem
0	0	5.8	6.3	2.8	3.4
0.02 (5 ppm)	5	5.9	6.0	2.8	3.3
0.09 (20 ppm)	20	5.6	6.1	2.6	3.3
0.27 (60 ppm)	60	5.6	5.9*	2.7	3.2*

*Statistically different from control mean by Dunnett's test, alpha = 0.05.

Urinalysis: no statistical differences between exposed and control rats were observed.

Terminal sacrifice body and organ weights: the mean final body weights of exposed male and female rats did not differ from the respective control values. The mean absolute and relative weights of heart, kidneys, liver and testes from exposed rats were similar to control values. The main absolute brain weight of female rats exposed to 0.27 mg/L/6h/day (60 ppm) was statistically decreased from the mean control value.

Final body weights. Brain Weights

Concentration (mg/L/6h/day)	**Final Body Wt.**		**Brain**			
	Male	**Fem**	**Male**	**Fem**	**Male**	**Fem**
	(g)	**(g)**	**(g)**	**(g)**	**(g/100)**	**(g/100)**
0	377.0	261.6	2.059	1.869	0.551	0.720
0.02 (5 ppm)	363.7	262.9	2.078	1.871	0.579	0.719
0.09 (20 ppm)	357.8	262.3	2.051	1.875	0.579	0.719
0.27 (60 ppm)	365.1	265.8	2.048	1.841*	0.564	0.702

*Statistically different from control mean by Dunnett's test, alpha = 0.05.

Gross Pathology: there were no gross pathologic observations indicative of apparent exposure-related effects.
Histopathology: statistically identified exposure-related effects occurred in nasal tissues of male and female rats exposed to 0.27 mg/L/6h/day (60 ppm) and were unilateral or bilateral decreased thickness of olfactory epithelium, unilateral or bilateral erosions of olfactory epithelium, and unilateral or bilateral submucosal fibrosis. Decreased thickness of olfactory epithelium was the most prevalent microscopic change in both male and female rats.

A variety of other inflammatory, degenerative, and/or hyperplastic, microscopic changes occurred in portions of the respiratory epithelium from some animals in all exposure groups and controls.

Statistically Identified Microscopic Changes in Nasal Tissues and Anatomic Level of Occurrence in Rats Exposed to 0.27 mg/L/6h/day (60 ppm) Telone II Soil Fumigant for 2 Years

Microscopic Change	**# Affected/ # Examined**	**%**	**Anatomic Level [a]**			
			1	2	3	4
Males 0.27 mg/L/6h/day (60 ppm)						
Decreased thickness olfactory epithelium	20/50	40	0	17	15	10
Erosions of olfactory epithelium	15/50	30	0	12	14	10
Submucosal fibrosis	6/50	12	0	4	5	6
Females 0.27 mg/L/6h/day (60 ppm)						
Decreased thickness olfactory epithelium	15/49	31	0	12	8	5
Erosions of olfactory epithelium	6/49	12	0	5	4	4
Submucosal fibrosis	2/49 [b]	4	0	0	1	1

[a]Any given animal may have 1 or more levels affected. Level 1 = most anterior, obtained just posterior to the incisor teeth; Level 2= obtained at the incisive papilla; Level 3 = obtained at level of second palatal ridge; Level 4 = most posterior, obtained at level of first upper molar teeth.

[b]Not statistically identified.

Histopathological findings in nasal tissues in tw0-year rat inhalation study showing grade of lesions

HISTOPATHOLOGIC OBSERVATIONS● - 2 YEARS

SEX		MALES				FEMALES			
EXPOSURE CONCENTRATION IN PPM		0	5	20	60	0	5	20	60
NUMBER OF RATS EXAMINED		50	50	50	50	50	50	50	50
NASAL TISSUES (CONTINUED)									
EROSION(S), RESPIRATORY EPITHELIUM, BILATERAL, MULTIFOCAL:	- SEVERE	1	0	0	0	0	0	0	0
EROSION(S), NASOLACRIMAL DUCT, UNILATERAL, MULTIFOCAL:	- SEVERE	0	0	0	0	1	0	0	0
FIBROSIS, SUBMUCOSA, UNILATERAL, MULTIFOCAL:	- MODERATE	0	0	0	1	0	0	0	0
FIBROSIS, SUBMUCOSA, BILATERAL, MULTIFOCAL:	- SLIGHT	0	0	0	3	0	0	0	1
FIBROSIS, SUBMUCOSA, BILATERAL, MULTIFOCAL:	- MODERATE	0	0	0	2	0	0	0	1
HYPERPLASIA, RESPIRATORY EPITHELIUM, UNILATERAL, FOCAL:	- SLIGHT	1	0	0	1	0	0	0	1
HYPERPLASIA, RESPIRATORY EPITHELIUM, UNILATERAL, MULTIFOCAL	- SLIGHT	1	1	1	0	0	2	1	0
HYPERPLASIA, RESPIRATORY EPITHELIUM, UNILATERAL, MULTIFOCAL	- MODERATE	0	3	4	1	0	0	0	0
HYPERPLASIA, RESPIRATORY EPITHELIUM, BILATERAL, MULTIFOCAL:	- SLIGHT	0	0	1	1	0	0	0	1
HYPERPLASIA, RESPIRATORY EPITHELIUM, BILATERAL, MULTIFOCAL:	- MODERATE	0	0	0	1	0	0	0	0
HYPERPLASIA, NASOLACRIMAL DUCT, UNILATERAL, FOCAL:	- SLIGHT	0	0	1	0	0	0	0	1
HYPERPLASIA - ADENOMATOUS, RESPIRATORY EPITHELIUM, BILATERAL, MULTIFOCAL:	- MODERATE	0	0	0	0	0	0	0	1
INFLAMMATION - CHRONIC, SUBMUCOSA, BILATERAL, MULTIFOCAL:	- SLIGHT	0	0	0	0	0	1	0	0
INFLAMMATION - CHRONIC ACTIVE, RESPIRATORY EPITHELIUM, UNILATERAL, FOCAL:	- SLIGHT	0	0	0	0	0	0	0	1

● DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.
- INDICATES NOT APPLICABLE.

- DOW CONFIDENTIAL -

SEX	MALES				FEMALES			
EXPOSURE CONCENTRATION IN PPM	0	5	20	60	0	5	20	60
NUMBER OF RATS EXAMINED	50	50	50	50	50	50	50	50
NASAL TISSUES (CONTINUED)								
INFLAMMATION - CHRONIC ACTIVE, RESPIRATORY EPITHELIUM, UNILATERAL, MULTIFOCAL: - SLIGHT	0	0	1	0	0	0	0	0
INFLAMMATION - CHRONIC ACTIVE, RESPIRATORY EPITHELIUM, UNILATERAL, MULTIFOCAL: - MODERATE	0	0	1	0	0	0	0	0
INFLAMMATION - CHRONIC ACTIVE, RESPIRATORY EPITHELIUM, UNILATERAL, MULTIFOCAL: - SEVERE	0	0	0	0	2	0	0	0
INFLAMMATION - CHRONIC ACTIVE, RESPIRATORY EPITHELIUM, BILATERAL, MULTIFOCAL: - SLIGHT	0	0	1	0	0	0	0	0
INFLAMMATION - CHRONIC ACTIVE, RESPIRATORY EPITHELIUM, BILATERAL, MULTIFOCAL: - MODERATE	2	0	0	0	0	0	0	0
INFLAMMATION - CHRONIC ACTIVE, SUBMUCOSA, UNILATERAL, FOCAL : - SLIGHT	0	0	0	2	0	0	0	0
INFLAMMATION - CHRONIC ACTIVE, SUBMUCOSA, UNILATERAL, MULTIFOCAL: - SLIGHT	0	3	1	1	0	2	2	3
INFLAMMATION - CHRONIC ACTIVE, SUBMUCOSA, UNILATERAL, MULTIFOCAL: - MODERATE	0	3	4	2	0	0	0	0
INFLAMMATION - CHRONIC ACTIVE, SUBMUCOSA, BILATERAL, MULTIFOCAL: - SLIGHT	1	0	0	3	0	0	0	1
INFLAMMATION - CHRONIC ACTIVE, SUBMUCOSA, BILATERAL, MULTIFOCAL: - MODERATE	2	0	0	1	0	0	0	1
INFLAMMATION - CHRONIC ACTIVE, NASOLACRIMAL DUCT, UNILATERAL, FOCAL: - SLIGHT	0	0	0	0	0	0	0	1
INFLAMMATION - CHRONIC ACTIVE, NASOLACRIMAL DUCT, UNILATERAL, MULTIFOCAL: - SLIGHT	0	1	1	0	0	0	0	1
INFLAMMATION - CHRONIC ACTIVE, NASOLACRIMAL DUCT, UNILATERAL, MULTIFOCAL: - MODERATE	0	0	0	0	0	1	0	0

@ DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.
- INDICATES NOT APPLICABLE. - DOW CONFIDENTIAL -

SEX	MALES				FEMALES			
EXPOSURE CONCENTRATION IN PPM	0	5	20	60	0	5	20	60
NUMBER OF RATS EXAMINED	50	50	50	50	50	50	50	50
NASAL TISSUES (CONTINUED)								
INFLAMMATION - CHRONIC ACTIVE, NASOLACRIMAL DUCT, UNILATERAL, MULTIFOCAL: - SEVERE	0	0	0	0	3	0	0	0
INFLAMMATION - SUBACUTE, OLFACTORY EPITHELIUM, BILATERAL, MULTIFOCAL: - MODERATE	0	0	0	1	0	0	0	0
INFLAMMATION - SUBACUTE, RESPIRATORY EPITHELIUM, BILATERAL, MULTIFOCAL: - MODERATE	0	0	0	1	0	0	0	0
INFLAMMATION - SUPPURATIVE, OLFACTORY EPITHELIUM, BILATERAL , MULTIFOCAL: - SLIGHT	0	0	0	1	0	0	0	0
INFLAMMATION - SUPPURATIVE, RESPIRATORY EPITHELIUM, BILATERAL, MULTIFOCAL: - SLIGHT	0	0	0	1	0	0	0	0
INFLAMMATION - SUPPURATIVE, NASOLACRIMAL DUCT, UNILATERAL, MULTIFOCAL: - SLIGHT	0	0	0	0	0	0	0	1
METAPLASIA - SQUAMOUS, RESPIRATORY EPITHELIUM, UNILATERAL, FOCAL: - SLIGHT	0	1	1	1	0	0	0	2
METAPLASIA - SQUAMOUS, RESPIRATORY EPITHELIUM, UNILATERAL, MULTIFOCAL: - SLIGHT	0	0	0	0	0	1	1	0
METAPLASIA - SQUAMOUS, RESPIRATORY EPITHELIUM, BILATERAL, MULTIFOCAL: - SLIGHT	0	0	1	0	0	0	0	0
NECROSIS, RESPIRATORY EPITHELIUM, BILATERAL, MULTIFOCAL: - MODERATE	1	0	0	0	0	0	0	0
THROMBUS - ACUTE OR RECENT, SUBMUCOSA, BILATERAL, MULTIFOCAL:	3	0	1	0	0	1	0	1
THROMBUS - ACUTE OR RECENT, SUBMUCOSA, BILATERAL, DIFFUSE:	1	0	0	0	0	0	0	0
EXUDATE, LUMEN, UNILATERAL, FOCAL:	1	0	4	1	2	1	2	0
EXUDATE, LUMEN, UNILATERAL, MULTIFOCAL:	0	5	0	2	0	1	0	1

@ DATA ARE THE NUMBER OF ANIMALS WITH THE SPECIFIED OBSERVATION.
- INDICATES NOT APPLICABLE. - DOW CONFIDENTIAL -

SEX	MALES				FEMALES			
EXPOSURE CONCENTRATION IN PPM	0	5	20	60	0	5	20	60
NUMBER OF RATS EXAMINED	50	50	50	50	50	50	50	50
NASAL TISSUES (CONTINUED)								
EXUDATE, LUMEN, BILATERAL, MULTIFOCAL:	2	0	2	1	0	0	0	2

Historical control values for nasal epithelial histopathology.

Relevant HCD for Nasal Effects in F344 Rats (Only one study available)		
Study Completion Year: 1986	Male	Female
# exanimated	50	49
Nasal Effect		
Lacrimal duct, mononuclear infiltration	31	30
Tooth, degeneration/alveoitis	9	6
Lacrimal duct, cyst (s)	1	0
Inflammation, chronic active	45	48
Erosion/ulceration	2	1
Lacrinal duct, chronic active inflammation	13	4
Microthrombus	3	1
Focal epithelial hypertrophy/hyperplasia	1	0
Foreign haterial	1	4
Goblet cells, hypertrophy	1	0
Squamous change	5	3
Atrophy, nasal epithelium	1	0
Squamous inclusion cyst	0	1

Statistically identified microscopic changes were observed in the heart, kidneys, liver and tongue. Chronic myocardial inflammation was statistically decreased in male rats exposed to 0.27 mg/L/6h/day (60 ppm) which was considered to be due to biological variability. Bilateral diffuse severe glomerulonephropathy was statistically decreased in female rats exposed to 0.02 or 0.09 mg/L/6h/day (5 or 20 ppm). Male rats exposed to 0.02 mg/L/6h/day (5 ppm) had a statistically identified increase in periportal fibrosis (all grades of severity combined) and foci of altered cells (moderate) in the liver. Female rats exposed to 0.27 mg/L/6h/day (60 ppm) had a statistically identified decrease in multifocal mineralization of blood vessels in the tongue.

Statistically identified histopathologic observations-2 year

Sex	Males				Females			
Exposure concentration (mg/L/6h/day)	**0**	**0.02 (5 ppm)**	**0.09 (20 ppm)**	**0.27 (60 ppm)**	**0**	**0.02 (5 ppm)**	**0.09 (20 ppm)**	**0.27 (60 ppm)**
Number of animals examined	50	50	50	50	50	50	50	50
ADRENALS (number examined)	50	49	50	50	50	50	50	50
Cellular infiltration, medula, bilateral multifocal								
Number of animals with observation	3	0	0	0T	0	0	1	0
Lesion rate	6%	0%	0%	0%	0%	0%	2%	0%

Sex	Males				Females			
Exposure concentration (mg/L/6h/day)	**0**	**0.02 (5 ppm)**	**0.09 (20 ppm)**	**0.27 (60 ppm)**	**0**	**0.02 (5 ppm)**	**0.09 (20 ppm)**	**0.27 (60 ppm)**
Number of animals examined	50	50	50	50	50	50	50	50
Pairwise p-value		0.124	0.121	0.121				
HEART (number examined)	50	24	21	50	50	24	13	50
Inflammation-chronic,myocardium,focal or multifocal								
Number of animals with observation	5	0	1	0*	2	0	0	1
Lesion rate	10%			0%	4%			2%
Pairwise p-value				0.033				
KIDNEYS (number examined)	50	50	50	50	50	50	50	50
Glomerolonephropathy, bilateral, diffuse, severe								
Number of animals with observation	15	15	15	10	9	1*	2*	7
Lesion rate	30%	30%	30%	20%	18%	2%	4%	14%
Pairwise p-value						0.010	0.028	
LIVER (number examined)	50	50	50	50	50	50	50	50
Fibrosis, periportal-any degree of severity								
Number of animals with observation	40	47*	44	34	17	26	26	14
Lesion rate	80%	94%	88%	68%	34%	52%	52%	28%
Pairwise p-value		0.037	0.207	0.127				
Congestion, sinusoids, diffuse								
Number of animals with observation	2	4	2	2	5	4	2	0*T
Lesion rate	4%	8%	4%	4%	10%	8%	4%	0%
Pairwise p-value						0.500	0.217	0.033
Focus (I) or altered cells, hepatocellular moderate								
Number of animals with observation	2	10*	2	2	17	13	22	15
Lesion rate	4%	20%	4%	4%	34%	26%	44%	30%
Pairwise p-value		0.016	0.610	0.610				

Sex	Males				Females			
Exposure concentration (mg/L/6h/day)	**0**	**0.02 (5 ppm)**	**0.09 (20 ppm)**	**0.27 (60 ppm)**	**0**	**0.02 (5 ppm)**	**0.09 (20 ppm)**	**0.27 (60 ppm)**
Number of animals examined	50	50	50	50	50	50	50	50
Infarct, focal								
Number of animals with observation	0	1	5*	0	0	0	0	0
Lesion rate	0%	2%	10%	0%	0%	0%	0%	0%
Pairwise p-value		0.500	0.033					
Necrosis, hepatocellular-any degree of severity								
Number of animals with observation	7	7	11	0	1	7*	4	0
Lesion rate	14%	14%	22%	0%	2%	14%	8%	0%
Pairwise p-value						0.033	0.179	0.500
NASAL TISSUES (number examined)	50	50	50	50	50	50	50	49
Decreased thickness, olfactory epithelium, unilateral of bilateral- any degree of severity								
Number of animals with observation	0	1	1	20	0	0	0	15*
Lesion rate	0%	2%	2%	40%	0%	0%	0%	31%
Pairwise p-value		0.500	0.500	0.000				0.000
Erosion, olfactory epithelium, unilateral or bilateral- any degree of severity								
Number of animals with observation	0	0	1	15*	0	0	0	6*T
Lesion rate	0%	0%	0%	30%	0%	0%	0%	12%
Pairwise p-value			0.500	0.000				0.017
Fibrosis, Submucosa, unilateral or bilateral- any degree of severity								
Number of animals with observation	0	0	0	6*T	0	0	0	2
Lesion rate	0%	0%	0%	12%	0%	0%	0%	4%
Pairwise p-value				0.018				
TONGUE (number examined)	50	23	19	50	50	25	13	49

Sex	Males				Females			
Exposure concentration (mg/L/6h/day)	**0**	**0.02 (5 ppm)**	**0.09 (20 ppm)**	**0.27 (60 ppm)**	**0**	**0.02 (5 ppm)**	**0.09 (20 ppm)**	**0.27 (60 ppm)**
Number of animals examined	50	50	50	50	50	50	50	50
Mineralization, blood vessels, multifocal								
Number of animals with observation	7	3	2	5	5	3	0	0*
Lesion rate	14%			10%	10%			0%
Pairwise p-value								0.033

*Statistically identified difference from control mean by Yate´s Chi-Square pairwise test. alpha= 0.05

T Linear Trend by Cochran-Armitage Linear Trend Test, alpha=0.02, two-side.

Histopathological findings in lungs

HISTOPATHOLOGIC OBSERVATIONS● - 2 YEARS

SEX	MALES				FEMALES			
EXPOSURE CONCENTRATION IN PPM	0	5	20	60	0	5	20	60
NUMBER OF RATS EXAMINED	50	50	50	50	50	50	50	50
LUNGS (NO. OF TISSUES EXAMINED)	50	50	50	50	50	50	50	50
WITHIN NORMAL LIMITS:	29	25	27	28	32	26	34	32
ATELECTASIS, ALVEOLI, MULTIFOCAL:	0	0	0	0	0	1	1	0
CHRONIC PASSIVE CONGESTIVE CHANGES, MULTIFOCAL:	1	0	0	0	0	0	0	0
CHRONIC PASSIVE CONGESTIVE CHANGES, DIFFUSE:	0	0	0	0	2	0	0	1

SEX	MALES				FEMALES			
EXPOSURE CONCENTRATION IN PPM	0	5	20	60	0	5	20	60
NUMBER OF RATS EXAMINED	50	50	50	50	50	50	50	50
LUNGS (CONTINUED)								
CONGESTION, BLOOD VESSELS, MULTIFOCAL:	0	0	0	0	0	0	1	0
CONGESTION, BLOOD VESSELS, DIFFUSE:	3	4	1	3	8	6	1	4
EDEMA, ALVEOLI, MULTIFOCAL:	1	1	0	1	3	1	0	1
FIBROSIS, INTERSTITIUM, MULTIFOCAL:	0	2	0	0	2	0	1	0
GRANULOMA(S) - FOREIGN BODY, ALVEOLI, MULTIFOCAL:	0	0	0	0	1	0	0	0
GRANULOMA(S) - MICRO, ALVEOLI, FOCAL:	0	0	0	0	0	0	0	1
HEMORRHAGE, ALVEOLI, FOCAL:	0	0	0	0	0	0	0	1
HEMORRHAGE, ALVEOLI, MULTIFOCAL:	1	0	0	1	1	0	0	1
HYPERPLASIA, PERIBRONCHIOLAR LYMPHOID TISSUE, MULTIFOCAL:	0	0	1	0	1	1	0	0
HYPERPLASIA, PERIBRONCHIOLAR LYMPHOID TISSUE, DIFFUSE:	0	0	0	0	0	1	0	0
HYPERPLASIA, PNEUMOCYTES, FOCAL:	1	1	2	0	0	0	1	2
HYPERPLASIA, PNEUMOCYTES, MULTIFOCAL:	1	1	1	0	0	0	1	1
HYPERPLASIA - EPITHELIAL, BRONCHIOLES, MULTIFOCAL:	0	0	0	0	1	0	0	0
INFLAMMATION - ACUTE, INTERSTITIUM, MULTIFOCAL:	0	0	0	1	0	0	0	0
INFLAMMATION - ACUTE, INTERSTITIUM, DIFFUSE:	0	0	0	0	1	0	0	0
INFLAMMATION - CHRONIC ACTIVE, ALVEOLI, MULTIFOCAL:	0	1	1	0	2	0	0	0
INFLAMMATION - CHRONIC ACTIVE, INTERSTITIUM, MULTIFOCAL:	0	0	0	2	1	0	0	1

SEX	MALES				FEMALES			
EXPOSURE CONCENTRATION IN PPM	0	5	20	60	0	5	20	60
NUMBER OF RATS EXAMINED	50	50	50	50	50	50	50	50
LUNGS (CONTINUED)								
METAPLASIA - SQUAMOUS, BRONCHIOLES, MULTIFOCAL:	0	0	0	0	1	0	0	0
MINERALIZATION, ALVEOLI, MULTIFOCAL:	0	1	0	2	0	0	0	0
MINERALIZATION, BLOOD VESSELS, MULTIFOCAL:	0	1	0	3	0	0	0	0
THROMBUS - ACUTE OR RECENT, BLOOD VESSELS, MULTIFOCAL:	0	0	0	1	0	0	0	0
MACROPHAGES, ALVEOLI, FOCAL:	0	1	0	0	0	0	0	1
MACROPHAGES, ALVEOLI, MULTIFOCAL:	1	5	2	1	4	2	1	5
CELLULAR INFILTRATION, ALVEOLI, MULTIFOCAL:	0	0	0	0	1	0	0	0
CELLULAR INFILTRATION, INTERSTITIUM, MULTIFOCAL:	1	0	0	1	1	2	2	2
CELLULAR INFILTRATION, INTERSTITIUM, DIFFUSE:	14	16	16	13	6	12	10	5
ADENOMA, ALVEOLI, BENIGN, PRIMARY:	2	1	0	0	0	0	0	0
CARCINOMA, BRONCHIOLES, MALIGNANT, PRIMARY:	0	0	1	0	0	0	0	0
PARAFOLLICULAR CELL ADENOCARCINOMA, (THYROID), MALIGNANT, SECONDARY:	0	0	0	0	0	1	0	0
PHEOCHROMOCYTOMA, (ADRENAL), MALIGNANT, SECONDARY:	0	1	0	0	0	0	0	0
FIBROSARCOMA, (SKIN & SUBCUTIS), MALIGNANT, SECONDARY:	0	0	1	0	0	0	0	0
FIBROUS HISTIOCYTOMA, (MES TISSUES), MALIGNANT, SECONDARY:	0	1	0	0	0	0	0	0
FIBROUS HISTIOCYTOMA, (SKIN & SUBCUTIS), MALIGNANT, SECONDARY:	0	0	1	0	0	0	1	0
MESOTHELIOMA, (TESTES), MALIGNANT, SECONDARY:	0	0	1	0	0	0	0	0

There were no statistically identified increases in any tumor incidence in rats exposed to TELONE II soil fumigant. Male rats exposed to 0.27 mg/L/6h/day (60 ppm) had slight increase in benign subcutaneous fibromas (10% incidence versus 6% incidence in controls).

Incidence of Selected Fibromas in Male Rats Exposed to Telone II for 2 Years

	Exposure Concentration (mg/L/6h/day			
Skin and subcutis	**0**	**0.02 (5 ppm)**	**0.09 (20 ppm)**	**0.27 (60 ppm)**
Fibroma, subcutaneous, benign, primary	3	3	3	5
Mammary Gland				
Fibroma, benign, primary	0	1	0	0
Combined Total	3	4	3	5

Historical laboratory data from previous 2-year studies using Fischer 344 rats revealed a range of 2-22% for benign fibromas in control male rats (all routes of exposure). Taken into account inhalatory studies, HCD are avalaible only from 2 studies with values of 10 and 22 % for benign fibromas in control male rats.

Historical Control Fibromas in F-3444 rats.

Study	A	B	C	D	E	F	G	H	Overall Incidence

Study Type	Diet	Inhalation *		Diet		Drinking Water	Diet	Diet	Inhalation	Diet	
Year Reported	1982	1983		1983		1984	1984	1986	1986	1987	
Number of Controls	86	60M-59F	60M-62F	50	50	60	50	50	50	50	
MALES											
Mammary											
Fibroma(s) - one	1	0	0	3	1	3	6	6	0	0	20/566=3.5%
- two	0	0	0	0	0	0	1	0	0	0	1/566=0.2%
Mammary											
Fibroma - one or more											21/566=3.7%
Subcutaneous											
Fibroma(s) - one	1	6	8	3	1	0	1	0	11	1	32/566=5.7%
- two	0	0	10	0	0	0	0	0	0	0	1/566=0.2%
Subcutaneous											
Fibroma - one or more											33/566=5.8%
Total with Fibroma(s) - subcutaneous or mammary	2	6	9	6	2	3	8	6	11	1	54/566=9.5%
Total in %	2	10	15	12	4	5	16	12	22	2	2--22% range
FEMALES											
Mammary											
Fibroma - one	0	0	0	0	0	0	1	1	0	0	2/567=0.4%
Subcutaneous											
Fibroma - one	0	3	0	0	0	0	1	1	4	1	10/567=1.8%
Total with fibroma(s) -- subcutaneous or mammary	0	3	0	0	0	0	2	2	4	1	12/567=2/1%
Total in %	0	5	0	0	0	0	4	4	8	2	0-8% range

Repeated inhalation dose toxicity studies (mice)

The 90-day mouse inhalation study by Anonymous 73 (1984) is considered unacceptable because it tested a batch of 1,3-D known to contain epichlorohydrin, added as a stabilizing agent. Epichlorohydrin is an *in vitro* and *in vivo* genotoxicant, and will confound the test results. Therefore, the data from this study is not considered as acceptable for the assessment of the repeat dose toxicity of 1,3-D and is not further discussed.

Anonymous 55 (1985) was an interim study covering 6- and 12-month exposure periods and was supplementary to the later 2-year study (Anonymous 56, 1987). The report does not mention the use of HCD to put into context the findings of this interim study. Four groups of 10 male and 10 female B6C3F1 mice were exposed via whole body administration to target concentrations of 0, 0.02, 0.09 or 0.27 mg/L/6h/day (0, 5, 20 or 60 ppm) for 6 hours a day, 5 days a week, for 2 years. However, with the exceptions of the deficiencies listed above, the study was deemed acceptable, with reservations. The chronic NOAEL was set at 0.02 mg/L/6h/day (5 ppm) based on no effects at this concentration. The chronic LOAEL was set at 0.09 mg/L/6h/day (20 ppm or 0.09 mg/L) at which epithelial hyperplasia/hypertrophy of the nasal turbinates.

In Anonymous 56 (1987) (the effects shown under the section for carcinogenicity), the mortality rate in this 2-year study was not reported; however, the report mentions the use of HCD to contextualize the findings in the study. With the exceptions of the deficiencies listed above, the study was deemed acceptable, with reservations. The chronic NOAEL was set a 0.02 mg/L/6h/day (5 ppm) based on no effects at this concentration. The chronic LOAEL was set at 0.09 mg/L/6h/day (20 ppm at which nasal mucosa/urinary bladder lesions and stomach epithelial hyperplasia were reported.

Results

6-month exposure

At 6 months no changes in body weight, haematological examinations and clinical chemistry were observed. Decreased liver and kidney weights were present in male mice from the 0.27 mg/L/6h/day (60 ppm) exposure group. These weight changes were histologically associated with a decrease in the degree of vacuolation normally observed in hepatocytes and renal epithelial cells of the proximal convoluted tubules. Although equivocal decreases in liver an kidney weights were present in males form the 0.09 mg/L/6h/day (20 ppm) exposure group, similar histopathologic changes were not observed in these males or females at any level. Histological changes were also present in the urinary bladders of 1/10 male and 4/10 female mice from the 0.27 mg/L/6h/day (60 ppm) exposure group and were characterised by a moderate hyperplasia of the transitional epithelium. This hyperplastic reaction was occasionally accompanied by an inflammatory reaction in the lamina propria of the urinary bladder. Neither hyperplasia nor inflammatory lesions occurred in male or female mice from the lower exposure groups.
Focal hyperplasia and hypertrophy of the respiratory epithelium was histologically observed in the nasal turbinates of male and female mice exposed to 0.27 mg/L/6h/day (60 ppm) and males exposed to 0.09 mg/L/6h/day (20 ppm) TELONE II. This lesion was very slight to slight in degree, was located in only the most anterior section, and was most prominent in males from the 0.27 mg/L/6h/day (60 ppm) exposure group.

Organ Weights 6-Months--Males

Concentration (mg/L/6h/day)	**Body Weight**	**Kidneys**		**Liver**	
	(g)	(g)	(g/100)	(g)	(g/100)
0	30.5	0.561	1.838	1.632	5.345
0.02 (5 ppm)	31.1	0.551	1.770	1.634	5.244
0.09 (20 ppm)	29.7	0.522*	1.759	1.544	5.194
0.27 (60 ppm)	29.9	0.478*	1.598*	1.425*	4.760*

*Statistically different from control mean by Dunnett's test, alpha = 0.05.

Histopathologic Observations (6-Month-Nasal Tissue, Urinary Bladder, Liver, Kidney)

Sex	Males				Females			
Dose in mg/kg/day	**0**	**5**	**20**	**60**	**0**	**5**	**20**	**60**
Number of Mice Examined	10	10	10	10	10	10	10	10
Nasal Tissues (# of Tissues Examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Within normal limits:	9	10	6	0	10	10	10	2
Inflammation – acute -very slight	0	0	1	0	0	0	0	1
Hyperplasia and hypertrophy, respiratory epithelium, focal: -very slight	1	0	3	2	0	0	0	7
Hyperplasia and hypertrophy, respiratory epithelium, focal: -slight	0	0	0	8	0	0	0	0
Urinary Bladder (# of Tissues Examined)	**10**	**10**	**10**	**9**	**10**	**10**	**10**	**10**
Within normal limits:	1	2	0	0	1	1	1	0
Aggregate(s) of mononuclear (predominantly lymphoid) cells: -very slight	9	7	8	6	7	7	7	4
Aggregate(s) of mononuclear (predominantly lymphoid) cells: -slight	0	1	2	2	2	2	2	4
Hyperplasia, epithelial cells: -moderate	0	0	0	1	0	0	0	4
Inflammation – subacute to chronic: -very slight	0	0	0	0	0	0	0	1
Inflammation – subacute to chronic: -slight	0	0	0	1	0	0	0	0
Inflammation – subacute to chronic: -moderate	0	0	0	0	0	0	0	1
Liver (# of Tissues Examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Within normal limits:	7	10	7	2	4	7	7	7
Vacuolation – decreased, hepatocellular: -slight	1	0	0	7	0	0	0	0
Extramedullary hematopoiesis: -slight	0	0	0	0	0	0	1	0
Focus(I) of altered cells, hepatocellular, focal:	2	0	1	0	1	0	0	0
Aggregates of re cells frequently adjacent to degenerative or necrotic hepatocytes, focal: -very slight	1	0	1	4	5	1	3	3
Aggregates of re cells frequently adjacent to degenerative or necrotic hepatocytes, multifocal: -very slight	0	0	1	1	0	2	0	0
Kidneys (# of Tissues Examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Within normal limits:	6	8	6	1	10	10	8	9
Vacuolation – decreased, convoluted tubule - proximal: -slight	4	1	2	9	0	0	0	0
Atrophy, individual nephron(s), focal: -very slight	0	1	3	0	0	0	1	1
Atrophy, individual nephron(s), multifocal: -very slight	0	0	0	1	0	0	0	0
Glomerulonephropathy: -moderate	0	0	0	0	0	0	1	0

12-month exposure

Two males in the 0 mg/L/6h/day (0 ppm) and one male in the 0.09 mg/L/6h/day (20 ppm) exposure group died unexpectedly during the study. These deaths were not attributed to TELONE II exposure. The only in-life observations interpreted to be treatment-related were slight decreases in body weight in male and female mice exposed to 0.27 mg/L/6h/day (60 ppm) TELONE II during the last months of exposure.
Hyperplastic and hypertrophic lesions involving the respiratory epithelium of the nasal turbinates were present in male and female mice exposed to 0.27 mg/L/6h/day (60 ppm) and males exposed to 0.09 mg/L/6h/day (20 ppm) TELONE II. These changes were similar to those noted after 6 months of exposure.

Male mice exposed to 0.27 mg/L/6h/day (60 ppm) TELONE II had decreased liver and kidney weights which were again associated with a decrease in the degree of vacuolation normally observed in hepatocytes and renal epithelial cells. This decrease in vacuolation in hepatocytes was interpreted to reflect a decrease in hepatic glycogen. A slight decrease in the relative liver weight was again noted in males from the 0.09 mg/L/6h/day (20 ppm) exposure group, however, this was not associated with a histopathologic change. Female mice did not have similar liver or kidney changes at any level.

The mucosa of the urinary bladder of a few female mice exposed to TELONE II was thickened. Hyperplasia of the transitional epithelium of the urinary bladder, similar to that noted at 6 months, was histologically observed in the majority of females exposed to 0.27 mg/L/6h/day (60 ppm) and in 1/10 females in the 0.09 mg/L/6h/day (20 ppm) exposure group. This hyperplastic reaction was characterised by a simple thickening of the epithelium and involved a variable amount of the mucosa, but primarily occurred in the area of the anterior ventral wall. Focal papillary hyperplasia, epithelial pigmentation, and vacuolization were also occasionally present. In addition to the epithelial changes, the lamina propria underlying the hyperplastic epithelium frequently contained inflammatory cells and prominent fibroblasts, connective tissue and vasculature.

Organ Weights 12-Months--Males

Concentration (mg/L/6h/day)	**Body Weight**	**Kidneys**		**Liver**	
	(g)	(g)	(g/100)	(g)	(g/100)
0	31.1	0.592	1.899	1.681	5.390
0.02 (5 ppm)	31.1	0.604	1.941	1.596	5.133
0.09 (20 ppm)	30.7	0.593	1.932	1.534	4.989*
0.27 (60 ppm)	30.0	0.513*	1.710*	1.381*	4.599*

*Statistically different from control mean by Dunnett's test, alpha = 0.05.

Histopathologic Observations (12-Month--Nasal Tissue, Urinary Bladder, Liver, Kidney)

Sex	**Males**				**Females**			
Dose in mg/kg/day	**0**	**5**	**20**	**60**	**0**	**5**	**20**	**60**
Number of Mice Examined	10	10	10	10	10	10	10	10
Nasal Tissues (# of Tissues Examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Within normal limits:	9	9	3	0	9	9	9	1
Decreased thickness, olfactory epithelium, focal: -very slight	0	0	0	0	1	0	0	0
Decreased thickness, olfactory epithelium, multifocal: -very slight	0	0	0	0	0	0	0	2
Inflammation – acute: -very slight	0	1	0	0	1	1	0	1
Inflammation – subacute to chronic: - very slight	0	0	0	0	0	0	1	1

Hyperplasia and hypertrophy, respiratory epithelium, focal: -very slight	1	0	7	3	0	0	0	8
Hyperplasia and hypertrophy, respiratory epithelium, focal: -slight	0	0	0	7	0	0	0	0
Urinary Bladder (# of Tissues Examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Within normal limits:	5	3	2	3	0	0	0	0
Aggregate(s) of mononuclear (predominately lymphoid) cells: -very slight	5	7	8	7	8	6	7	3
Aggregate(s) of mononuclear (predominately lymphoid) cells: -slight	0	0	0	0	2	4	3	2
Hyperplasia, epithelial cells: -slight	0	0	0	0	0	0	1	5
Hyperplasia, epithelial cells: -moderate	0	0	0	0	0	0	0	4
Inflammation – subacute to chronic: -very slight	0	0	0	0	0	0	0	1
Inflammation – subacute to chronic: -slight	0	0	0	0	0	0	0	3
Liver (# of Tissues Examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Within normal limits:	4	7	8	3	5	1	5	6
Vacuolation – decreased, hepatocellular: -slight	0	0	0	5	0	0	0	0
Vacuolation – decreased, hepatocellular: -severe	1	0	1	0	0	0	0	0
Aggregate(s) of mononuclear (predominatley lymphoid) cells: -slight	0	0	0	0	0	1	0	1
Focus(I) of altered cells, hepatocellular, focal:	2	1	0	1	0	0	0	0
Increased mitotic figures, hepatocellular; -very slight	1	0	0	0	0	0	0	0
Mineralization, focal: -very slight	0	0	0	0	0	1	0	0
Mineralization, multifocal: -very slight	0	0	0	0	1	0	0	0
Necrosis- individual cell(s), hepatocellular, multifocal: -very slight	1	0	0	0	0	0	0	0
Pigment-laden macrophages, focal: -very slight	0	0	0	0	0	1	0	0
Aggregates of re cells frequently adjacent to degenerative or necrotic hepatocytes, focal: -very slight	1	1	0	2	2	3	2	2
Aggregates of re cells frequently adjacent to degenerative or necrotic hepatocytes, multifocal: -very slight	0	1	0	0	2	4	3	0
Adenoma, hepatocellular, benign, primary:	1	1	1	0	0	0	0	1
Carcinoma, hepatocellular, malignant, primary, metastasis:	1	0	0	0	0	0	0	0
Kidneys (# of Tissues Examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Within normal limits:	4	4	5	0	7	9	7	8
Vacuolation – decreased, convoluted tubule - proximal: -slight	1	0	0	4	0	0	0	0
Vacuolation – decreased, convoluted tubule - proximal: -moderate	0	0	0	5	0	0	0	0
Atrophy, individual nephron(s), focal: -very slight	4	2	4	4	3	1	1	2
Atrophy, individual nephron(s), multifocal: -very slight	1	4	1	3	0	0	0	0
Glomerulonephropathy: -slight	0	0	0	0	0	0	1	0

Glomerulonephropathy: -moderate	0	0	0	0	0	0	1	0
Mineralization, tubule(s): -very slight	0	2	0	2	0	0	0	0

2-year exposure

In this study, the following effects were noted:

0.09 mg/L/6hr/day (20 ppm)—hyperplasia of the transitional epithelium of the urinary bladder in both sexes, hyperplasia and hypertrophy of the respiratory epithelium of the nasal mucosa in females only,

0.27 mg/L/6hr/day (60 ppm**)**—hyperplasia of the transitional epithelium of the urinary bladder in both sexes, hyperplasia and hypertrophy of the respiratory epithelium of the nasal mucosa in both sexes, degeneration of olfactory epiethelium of the nasal mucosa in both sexes, hyperplasia of the mucosa of the nonglandular portion of the stomach in males, decreased incidence of vacuolation of the proximal tubular epithelial cells of the kidneys of males, decreased incidence of vacuolation of hepatocytes of females, and an increase in the incidence of benign lung tumors (bronchioloalveolar adenomas) in males.

2-year mouse Gross Pathologic Observations (Lungs, Lacrimal Glands, Uterus, Urinary Bladder)

Sex	***Males***				***Females***			
Exposure Concentration (ppm)	**0**	**5**	**20**	**60**	**0**	**5**	**20**	**60**
Number of Mice Examined	50	50	50	50	50	50	50	50
Lungs								
Within normal limits	43	44	39	32	42	45	44	46
Atelectasis	0	0	0	0	0	2	1	0
Atelectasis, focal	0	0	1	0	0	0	0	0
Atelectasis, right apical lobe	0	0	0	0	0	0	0	1
Congestion	0	0	0	1	2	0	0	0
Dark	0	0	0	0	0	0	0	1
Focus – dark	0	0	0	1	0	0	0	0
Focus - pale, multifocal	0	0	0	0	1	0	0	1
Focus - pale elevated	2	2	1	2	1	0	2	0
Focus - pale elevated, right cardiac lobe, focal	0	0	0	0	1	0	0	0
Mass/nodule--probably metastatic tumor, multifocal	0	1	0	0	1	0	0	0
Mass/nodule	5	3	8	13	2	3	3	2
Mass/nodule (two)	0	0	1	1	0	0	0	0
Lacrimal/Harderian Gland(s)								
Within normal limits	50	47	44	47	48	48	49	49
Mass/nodule	0	3	6	2	2	2	1	1
Mass/nodule (two)	0	0	0	1	0	0	0	0
Urinary Bladder								
Within normal limits	50	49	50	50	50	50	50	50
Focus - dark, wall	0	1	0	0	0	0	0	0
Urinary Bladder (Using dissecting Microscope on Fixed Distended Urinary Bladders								
Focus Dark (hemorrhage) serosa, focal	1	0	0	0	0	0	0	0
Roughened, irregular and opaque surface -slight	0	1	0	2	3	4	7	14
Roughened, irregular and opaque surface -moderate	0	0	0	3	0	1	11	14
Roughened, irregular and opaque surface -marked	0	0	0	1	0	0	2	2
Mass or nodule	0	1	0	0	0	0	1	0
Uterus								
Within normal limits					9	10	10	14
Hyperplasia - cystic endometrial					39	38	39	32
Distended - with clear fluid, unilateral					1	0	0	1
Mass/nodule					1	5	4	8

2-year mouse histopathological findings in the lungs

SEX		MALES				FEMALES			
EXPOSURE CONCENTRATION IN PPM		0	5	20	60	0	5	20	60
NUMBER OF MICE EXAMINED		50	50	50	50	50	50	50	50
LUNGS (NO. OF TISSUES EXAMINED)		50	50	50	50	50	50	50	50
WITHIN NORMAL LIMITS:		12	15	14	6	26	32	26	28
ABSCESS, FOCAL:		0	0	1	0	0	0	0	0
AGGREGATE(S) OF MONONUCLEAR (PREDOMINATELY LYMPHOID) CELLS, MULTIFOCAL:	- SLIGHT	1	5	0	5	6	7	9	8
AGGREGATE(S) OF MONONUCLEAR (PREDOMINATELY LYMPHOID) CELLS, MULTIFOCAL:	- MODERATE	0	0	0	0	0	1	2	0
ALVEOLAR HISTIOCYTOSIS, FOCAL:	- VERY SLIGHT	0	1	1	1	5	4	5	2
ALVEOLAR HISTIOCYTOSIS, FOCAL:	- SLIGHT	3	2	0	3	1	2	0	0
ALVEOLAR HISTIOCYTOSIS, MULTIFOCAL:	- VERY SLIGHT	1	0	1	1	1	0	0	1

HISTOPATHOLOGIC OBSERVATIONS● - 2 YEARS

SEX		MALES				FEMALES			
EXPOSURE CONCENTRATION IN PPM		0	5	20	60	0	5	20	60
NUMBER OF MICE EXAMINED		50	50	50	50	50	50	50	50
LUNGS (CONTINUED)									
ALVEOLAR HISTIOCYTOSIS, MULTIFOCAL:	- SLIGHT	1	1	3	0	2	2	4	0
ALVEOLAR HISTIOCYTOSIS, MULTIFOCAL:	- MODERATE	0	0	2	2	2	0	0	0
ALVEOLAR HISTIOCYTOSIS, DIFFUSE:	- MODERATE	0	0	0	0	0	0	0	1
HYPERPLASIA, ALVEOLI, FOCAL:		0	1	2	3	0	0	1	2
ADENOCARCINOMA, BRONCHIOLOALVEOLAR, MALIGNANT, PRIMARY, NO METASTASIS:		0	0	1	0	0	0	0	0
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY:		9	6	11	20	3	3	4	3
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY: (TWO)		0	0	2	2	0	0	1	0
ADENOMA, BRONCHIOLOALVEOLAR, BENIGN, PRIMARY: (THREE)		0	0	0	0	1	0	0	0
CARCINOMA, (LIVER), MALIGNANT, SECONDARY:		0	1	1	1	0	0	0	0

2-year mouse histopathology in the nasal tissues

HISTOPATHOLOGIC OBSERVATIONS● - 2 YEARS

SEX		MALES				FEMALES			
EXPOSURE CONCENTRATION IN PPM		0	5	20	60	0	5	20	60
NUMBER OF MICE EXAMINED		50	50	50	50	50	50	50	50
NASAL TISSUES (NO. OF TISSUES EXAMINED)		50	50	50	50	50	50	50	50
WITHIN NORMAL LIMITS:		30	29	30	0	22	23	6	0
DEGENERATION, OLFACTORY EPITHELIUM, FOCAL:	- VERY SLIGHT	5	5	5	1	4	6	10	2
DEGENERATION, OLFACTORY EPITHELIUM, MULTIFOCAL:	- VERY SLIGHT	0	0	1	0	0	0	0	1

SEX		MALES				FEMALES			
EXPOSURE CONCENTRATION IN PPM		0	5	20	60	0	5	20	60
NUMBER OF MICE EXAMINED		50	50	50	50	50	50	50	50
NASAL TISSUES (CONTINUED)									
DEGENERATION, OLFACTORY EPITHELIUM, MULTIFOCAL:	- MODERATE	0	0	0	0	1	0	0	0
DEGENERATION, OLFACTORY EPITHELIUM, BILATERAL:	- VERY SLIGHT	1	0	1	32	0	0	1	29
DEGENERATION, OLFACTORY EPITHELIUM, BILATERAL:	- SLIGHT	0	0	0	16	0	0	0	16
INFLAMMATION - SUPPURATIVE, FOCAL:	- SLIGHT	0	0	0	0	0	1	0	1
INFLAMMATION - SUPPURATIVE, NASOLACRIMAL DUCT, UNILATERAL:		1	0	0	0	0	0	0	0
CYST(S) WITH KERATINOUS DEBRIS, NASOLACRIMAL DUCT, UNILATERAL:		0	0	0	1	0	0	0	0
HYPERPLASIA AND HYPERTROPHY, RESPIRATORY EPITHELIUM, BILATERAL:	- VERY SLIGHT	5	1	4	38	4	4	28	39
HYPERPLASIA AND HYPERTROPHY, RESPIRATORY EPITHELIUM, BILATERAL:	- SLIGHT	0	0	0	10	0	0	0	10
DILATATION, AND AGGREGATES OF CELLULAR DEBRIS, SUBMUCOSAL GLANDS, FOCAL:	- VERY SLIGHT	12	14	14	7	17	19	20	10
DILATATION, AND AGGREGATES OF CELLULAR DEBRIS, SUBMUCOSAL GLANDS, MULTIFOCAL:	- VERY SLIGHT	2	2	1	3	4	3	5	8
GLANDULAR ATROPHY, MAXILLARY RECESS:	- MODERATE	1	1	0	0	1	1	0	0

2-year mouse histopathological findings in the bladder

SEX		MALES				FEMALES			
EXPOSURE CONCENTRATION IN PPM		0	5	20	60	0	5	20	60
NUMBER OF MICE EXAMINED		50	50	50	50	50	50	50	50
URINARY BLADDER (NO. OF TISSUES EXAMINED)		50	50	50	50	50	50	50	50
WITHIN NORMAL LIMITS:		10	10	6	6	2	2	0	0
AGGREGATE(S) OF MONONUCLEAR (PREDOMINATELY LYMPHOID) CELLS, SUBMUCOSA:	- VERY SLIGHT	36	36	39	28	41	36	32	19
AGGREGATE(S) OF MONONUCLEAR (PREDOMINATELY LYMPHOID) CELLS, SUBMUCOSA:	- SLIGHT	1	0	1	1	4	7	7	16
AUTOLYSIS:		3	2	2	3	3	4	2	5
HYPERPLASIA, MUCOSA:	- VERY SLIGHT	4	7	7	16	1	3	13	5
HYPERPLASIA, MUCOSA:	- SLIGHT	0	0	3	18	0	1	6	18
HYPERPLASIA, MUCOSA:	- MODERATE	0	0	0	2	0	0	0	19
HYPERPLASIA - NODULAR, MUCOSA:	- SLIGHT	0	0	1	0	0	0	0	0
HYPERPLASIA - NODULAR, MUCOSA:	- MODERATE	0	0	0	1	0	0	2	2
INFLAMMATION - CHRONIC:	- SLIGHT	0	0	0	0	0	1	0	7
INFLAMMATION - CHRONIC:	- MODERATE	0	0	0	0	0	0	2	0
INFLAMMATION - CHRONIC ACTIVE:	- MODERATE	0	0	0	2	0	0	4	0
INFLAMMATION - CHRONIC ACTIVE:	- SEVERE	0	0	0	0	0	0	0	1
MINERALIZATION, BLOOD VESSEL, SEROSA, FOCAL:	- SLIGHT	1	1	0	0	0	0	0	0
CARCINOMA, MALIGNANT, PRIMARY, NO METASTASIS:		0	0	0	0	0	0	2	0
URINARY BLADDER (CONTINUED)									
PAPILLARY ADENOMA, BENIGN, PRIMARY:		0	0	0	0	0	0	1	0
HEMANGIOMA, BENIGN, PRIMARY:		0	1	0	0	0	0	0	0

Summary and discussion of repeated dose toxicity by the inhalation route

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. Both factors have to be evaluated by weight of evidence and expert judgement.

Findings in the repeat dose studies by the inhalation route that are potentially relevant to the classification for STOT RE are summarised in the table below:

Species/ sex/ number Exposure route	**Duration of exposure and dose (mg/L/6 hr day)**	**Upper respiratory tract**	**Lower respiratory tract**	**Systemic effects**	**Study Reference**
Rat 15m/15f Whole body	2-week study 6h/d, 5d/wk, 9 exposures 0.0, 0.05, 0.27 or 0.68 mg/L/6hr/day	0.68 mg/L/6hr/day In both sexes bilateral diffuse degeneration of olfactory epithelium in all animals (grade moderate) and bilateral multifocal hyperplasia of respiratory epithelium in all animals (grade moderate), and in all animals bilateral multifocal exudate in the luman. 0.27 mg/L/6hr/day No adverse histopathological findings 0.05 mg/L/6hr/day No adverse histopathological findings	No adverse histopathological findings at any dose	0.68 mg/L/6hr/day Reduced bodyweight in both sexes 0.27 mg/L/6hr/day No adverse findings 0.05 mg/L/6hr/day No adverse findings	Anonymous 48 (1990)
Rat 5m/5f Nose only	2-week study 6h/d, 5d/wk 0.0, 0.14, 0.45, 1.36 mg/L/6hr/day	1.36 mg/L/6hr/day no findings no histopathology conducted 0.45 mg/L/6hr/day no findings no histopathology conducted 0.14 mg/L/6hr/day no findings no histopathology conducted	None (no histopathology conducted)	1.36 mg/L/6hr/day Reduced bodyweight in both sexes 0.45 mg/L/6hr/day Reduced bodyweight in both sexes 0.14 mg/L/6hr/day Reduced bodyweight in males	Anonymous 50 (1999)
Rat 10m/10f Whole body	90-day study, 6h/d, 5d/wk 0.0, 0.05, 0.14 or 0.41 mg/L/6hr/day	0.41 mg/L/6hr/day In both sexes bilateral multifocal degeneration of olfactory epithelium in all animals (grade slight) and bilateral multifocal hyperplasia of respiratory epithelium in all animals (grade slight) 0.14 mg/L/6hr/day No adverse histopathological findings 0.05 mg/L/6hr/day No adverse histopathological findings	No adverse histopathological findings at any dose	0.41 mg/L/6hr/day Reduced bodyweights in males. 0.14 mg/L/6hr/day No adverse findings 0.05 mg/L/6hr/day No adverse findings	Anonymous 49 (199
Rat 10m/10f Nose only	90-day study, 6h/d, 5d/wk 0.0, 0.05, 0.14 or 0.41 mg/L/6hr/day	0.41 mg/L/6hr/day No adverse histopathological findings 0.14 mg/L/6hr/day no findings no histopathology conducted	None	0.41 mg/L/6hr/day In males reduced bodyweight. In females increased absolute and relative liver weight.	Anonymous 51 (1999)

		0.05 mg/L/6hr/day no findings no histopathology conducted		0.14 mg/L/6hr/day no findings no histopathology conducted 0.05 mg/L/6hr/day no findings no histopathology conducted	
Rat 10m/10f Whole body	6-month 6h/d, 5d/wk 0, 0.02, 0.09, 0.27 mg/L/6hr/day	No adverse histopathological findings at any dose	No adverse histopathological findings at any dose	Slight bodyweight decrease at 0.27 mg/L/6hr/day No adverse histopathological findings at any dose	Anonymous 147 (1985)
Rat 10m/10f Whole body	1-year 6h/d, 5d/wk 0, 0.02, 0.09, 0.27 mg/L/6hr/day	No adverse histopathological findings at any dose	No adverse histopathological findings at any dose	Slight bodyweight decrease at mg/L/6hr/day No adverse histopathological findings at any dose	Anonymous 147 (1985)
Rat 50m/50f Whole body	2-year 6h/d, 5d/wk 0, 0.02, 0.09, 0.27 mg/L/6hr/day In DAR LOAEL set at 0.27 based on olfactory epithelium changes	0.27 mg/L/6hr/day Decreased thickness and erosion of olfactory epithelium In both sexes. Fibrosis of submucosa in nasal tissue in both sexes. Hyperplasia of respiratory epithelium both sexes (grade slight to moderate). Chronic multifocal inflammation of submucosa in both sexes (grade slight to moderate). Metaplasia of squamous epithelium in nasal tissue in both sexes (grade slight). 0.09 mg/L/6hr/day No adverse histopathological findings 0.02 mg/L/6hr/day No adverse histopathological findings	No adverse histopathological findings at any dose	0.27 mg/L/6hr/day Decreased body weights in both sexes 0.09 mg/L/6hr/day No adverse findings 0.02 mg/L/6hr/day No adverse findings	Anonymous 58, 1987
Mouse 10m/10f Whole body	6-month 6h/d, 5d/wk 0, 0.02, 0.09, 0.27 mg/L/6hr/day	0.27 mg/L/6hr/day Focal hypertrophy and hyperplasia of respiratory epithelium at 0.27 mg/L in both sexes (grade slight to very slight). 0.09 mg/L/6hr/day Focal hyperplasia and hypertrophy of respiratory epithelium in males (grade very slight) 0.02 mg/L/6hr/day No adverse histopathological findings 0.04 mg/L/6hr/day	No adverse histopathological findings at any dose	0.27 mg/L/6hr/day In males a reduction in absolute and relative heart, kidney and liver weight, and decreased vacuolation of liver and kidneys. In females in urinary bladder increased hyperplasia of epithelial cells (moderate) and increased inflammation (grade very slight to moderate) 0.09 mg/L/6hr/day No adverse findings 0.02 mg/L/6hr/day No adverse findings	Anonymous 55, 1985

		No adverse histopathological findings			
Mouse 10m/10f Whole body	1-year 6h/d, 5d/wk 0, 0.02, 0.09, 0.27 mg/L/6hr/day	0.27 mg/L/6hr/day Focal hyperplasia and hypertrophy of respiratory epithelium at 0.27 mg/L in both sexes (grade very slight to slight) 0.09 mg/L/6hr/day Focal hyperplasia and hypertrophy of respiratory epithelium in males (grade very slight). 0.02 mg/L/6hr/day No adverse findings	No adverse histopathological findings at any dose	0.27 mg/L/6hr/day In males a reduction in absolute and relative liver and kidney weight, and decreased vacuolation of liver and kidneys. In females in urinary bladder increased hyperplasia of epithelial cells (grade slight to moderate) and increased inflammation (grade very slight to slight). 0.09 mg/L/6hr/day No adverse findings 0.02 mg/L/6hr/day No adverse findings	Anonymous 55, 1985
Mouse 50m/50f Whole body	2-year 6h/d, 5d/wk 0, 0.02, 0.09, 0.27 mg/L/6hr/day	0.27 mg/L/6hr/day Bilateral degeneration of olfactory epithelium (grade very slight to slight) in both sexes. Bilateral hyperplasia and hypertrophy of respiratory epithelium (grade very slight to slight) in both sexes. 0.09 mg/L/6hr/day Bilateral hyperplasia and hypertrophy of respiratory epithelium (grade very slight to slight).	0.27 mg/L/6hr/day Focal hyperplasia of alveoli in both sexes and increased bronchioloaveolar adenomas in males. 0.09 mg/L/6hr/day No adverse findings 0.02 mg/L/6hr/day No adverse findings	0.27 mg/L/6hr/day Reduced body weight in both sexes, In males a reduction in absolute and relative heart. liver and kidney weight. Decreased vacuolation of liver in females. Decrease vacuolation of kidneys in males. Gross pathology roughened bladder with irregular and opaque surface (grade slight to marked) in both sexes. Hyperplasia of stomach in males. Urinary bladder increased hyperplasia of mucosa (grade very slight to moderate) in both sexes. Chronic inflammation of urinary bladder in both sexes (grade moderate in males and slight to severe in females). 0.09 mg/L/6hr/day Gross pathology in females: roughened bladder with irregular and opaque surface (grade slight to marked). Urinary bladder histopathology: increased hyperplasia of mucosa in both sexes	Anonymous 56, 1987

				(grade very slight to slight). Chronic inflammation of urinary bladder in females (grade moderate). 0.02 mg/L/6hr/day No adverse histopathological findings	

A chemical substance may induce systemic and/or local effects. For the inhalation studies conducted with 1,3-D, local effects include respiratory irritation in the upper and lower respiratory tract. It is proposed to classify 1,3-D for severe target organ toxicity following single exposure (STOT SE Category 3 H335) based on evidence of respiratory irritation both in animals and humans (see Section 10.11). Chemical substances that are respiratory irritants following single exposure are likely to also cause similar effects in the respiratory tract following repeated inhalation exposure.

As stated in the ECHA Guidance on the application of CLP criteria Section 3.9.2.5.1 substances classified as corrosive may cause severe toxicological effects following repeated exposure, especially in the lungs following inhalation exposure. Therefore in the case of 1,3-D which is a respiratory irritant, it has to be evaluated whether the effects in the respiratory tract following repeat dose inhalation studies is a reflection of true repeated exposure toxicity or just acute toxicity (in this case irritation).

One way to distinguish between these possibilities is to consider the dose that causes the toxicity. If the dose is more than half an order of magnitude lower than that causing acute effects then it could be considered to be a repeated-dose effect distinct from a single dose effect.

Upper respiratory tract irritation

Mechanisms of respiratory tract irritation are discussed in ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance (Version 6.0 July 2017pages 253-254). Typical effects of corrosion of the respiratory tract include destruction of the mucosa followed by proliferation of epithelial cells. Mild epithelial or endothelial injury without basement membrane damage, severe inflammation, or persistence of the inciting agent may be resolved by simple cellular regeneration. With more severe damage, a significant inflammation component may be elicited which may be followed by tissue destruction or fibrosis. Cytotoxic effects in the respiratory tract are comparable to dermal and eye irritation. These effects are characterised by inflammation, local infiltration with white blood cells, swelling, oedema, and there may also be haemorrhage, and eventual necrosis and other pathological changes.
Chronic irritation can lead to repeated episodes of cell proliferation in the affected tissues, and this may increase the risk of tumour development. The nature of effects depends on the substance and its primarily targeted region; the severity of effects depends on the concentration and duration of exposure.

Repeated-dose inhalation studies conducted on 1,3-D consistently show adverse histopathological findings in the upper respiratory tract consistent with respiratory irritation at the site of contact/entry. Lesions are typical of respiratory irritation including:

1. Decreased thickness and erosion/degeneration of the olfactory epithelium (Anon 58 (1987), Anon 56 (1987), Anon 51 (1999), Anon 49 (1991), Anon 48 (1990)) due to tissue damage from an irritant substance at the site of contact – at concentrations at an above 0.27 mg/L/6hr/day.
2. Chronic inflammation of the nasal submucosa (Anon 58 (1987)) – at concentrations at 0.27 mg/L/6hr/day.

3. Hyperplasia and/or hypertrophy of the upper respiratory epithelium (Anon 48 (1990), Anon 49 (1991), Anon 58 (1987), Anon 55 (1985), Anon 56 (1987)) as part of a repair mechanism triggered by tissue damage – at concentrations at an above 0.09 mg/L/6hr/day.
4. Fibrosis of submucosa in nasal tissue (Anon 58 (1987)) as a consequence of a repair response following severe tissue damage – at concentrations of 0.27 mg/L/6hr/day.
5. Metaplasia of squamous epithelium in nasal tissue (Anon 58 (1987)) as a consequence of repeated tissue damage – at concentrations of 0.27 mg/L/6hr/day.

Comparisons between acute and repeat dose effects can be made to clinical signs and adverse findings in the lower respiratory tract:

Lower respiratory tract irritation

Following repeat dose exposure the only adverse findings in the lungs were seen in the 2-year mouse study where there was focal hyperplasia of alveoli in both sexes and increased bronchioloalveolar adenomas in males at concentration of 0.27 mg/L/6hr/day. This dose is above the trigger dose for STOT RE classification. There were no adverse findings in the lungs in mice up to 1-year exposure (maximum dose 0.27mg/L/6hr/day). There were no adverse findings in the lungs in rat studies of up to 2 year duration (maximum dose 0.27 mg/L/6hr/day) or at doses up to 1.36 mg/L/6hr/day (in a 14 day study).

The dose causing findings in the lungs in the 2-year mouse study is ten times lower than the lowest concentration causing lung congestion and mortality following acute exposure (Anon. 12 (1987)) which was 3.1 mg/L/4hr exposure equivalent to 2.07 mg/L/6hr exposure (using Haber's law conversion), however toxicity findings following lifetime exposure at 0.27 mg/L/6hr/day are considerably milder compared to a lethal concentration, especially when total exposure is considered using Haber's law: a 2 year study of 6hr exposure 5 days a week (= 0.27 x 520 6hr exposures) is equivalent to a single oral exposure of 140.4mg/L/6hr/day thus there is evidence that repeated exposure does not cause increased damage in the lungs and is better tolerated compared to single acute exposure.

Clinical signs of respiratory irritation

Following repeat dose exposure there were no clinical signs of toxicity in any study up to the maximum concentration tested of 1.36 mg/L/6hr/day (2-week rat study, Anon. 50 (1999)). This dose is only slightly lower than doses causing clinical signs following a single exposure (1.6 mg/L/4hr exposure or 1.07 mg/L/6hr exposure using Haber's law conversion), thus there is evidence that repeated exposure does not cause increased clinical signs or respiratory irritation (lower respiratory rate and irregular respiration) in the lungs compared to single acute exposure.

In conclusion the effects in the respiratory system following repeat dose inhalation exposure to 1,3-D are consistent with respiratory irritation and there is no marked progression in severity or sensitivity compared to acute exposure, therefore classification for STOT SE Category 3 is sufficient to address the effects on the respiratory system and STOT RE is not triggered.

Systemic toxicity via inhalation exposure to 1,3-D

Systemic effects can occur following absorption via the respiratory tract. Most of the inhalation studies available use whole body exposure and since 1,3-D is a vapour there will be some condensation of the vapour on the animal fur leading to oral ingestion via grooming; therefore some systemic exposure will be via the oral route, resulting in an overall exposure likely to be higher than that estimated via the inhalation route alone.

Systemic toxicity reported in the repeat dose inhalation studies in general are minimal in nature, the most consistent effect being a moderate reduction in body weight compared to control animals and some minimal changes in organ weights and the LOAEL for these effects generally occur at doses higher than those triggering respiratory irritation. In the rat there were no specific or severe systemic effects.

The mouse was the most sensitive species to systemic effects and the urinary bladder was a target organ in the 6-month (females), 1-year (females) and 2-year studies (both sexes). Findings in the bladder were seen at doses of 0.27 mg/L/6hr/day in both sexes and at 0.09 mg/L/6hr/day in females following 2-year exposure and comprised of increased inflammation and aggregates of mononuclear cells accompanied by hyperplasia of epithelial cells, consistent with chronic irritation in the bladder. The grade of lesions was slight to very slight for mononuclear cell aggregation, very slight to moderate for inflammation, and very slight to moderate for hyperplasia. The lesions did not show a marked increase in incidence or severity following increased exposure duration. Findings in the bladder are discussed in detail in the carcinogenicity section (10.9.1). The urinary bladder was also a target organ in mice following oral exposure (Anon. 52 (1997), and NTP 1985). The NTP study is not relevant due to its impurity profile containing a genotoxic stabiliser. The other oral study is an 18-month study (Anon, 52 (1997)) with findings in the bladder in females at doses of 25 mg/kg bw/day. Since the mouse inhalation studies used whole body exposure and oral ingestion of the substance is likely to occur there is uncertainty whether the effects in the urinary bladder are derived via inhalation or oral absorption.

10.12.2 Conclusion on classification and labelling for STOT RE

Oral

Based on the evaluation of available experimental data from repeat dose oral toxicity studies in rats conducted using 1,3-D (mix of isomers), cis 1,3-D (cis isomer) and trans 1,3-D (trans isomer), these substances should be classified in accordance with the CLP Regulation as:

STOT RE category 2 – H373: ‘May cause damage to organs through prolonged or repeated exposure with the stomach as the target organ.

Inhalation

Based on the evaluation of available experimental data from repeat dose inhalation toxicity studies in rats and mice conducted using 1,3-D (mix of isomers), cis 1,3-D (cis isomer) and trans 1,3-D (trans isomer), classification is not considered to be applicable due to the following reasons:

- Of the inhalation studies available only two are nose-only (14-day and 90-day rat). Nose-only studies are the preferred method of inhalation exposure as this minimizes exposure or uptake by non-inhalation routes, giving a more reliable estimate of the test substance exposure. All other studies are whole body exposure where calculation of the absorbed dose may underestimate total exposure because vapour droplets of the test substance may condense onto animal fur leading to oral ingestion by grooming which may continue after the 6-hour inhalation exposure period. Therefore the whole-body studies including all studies in the mouse are confounded by an unquantified additional oral exposure in addition to inhalation exposure route.

- Findings in the upper respiratory tract are consistent with exposure to an irritant which is already reflected in the proposed classification for STOT SE Category 3 H335. Therefore classification for STOT RE is not warranted as repeated inhalation exposure does not show a significant increase in hazard or dose-sensitivity compared to acute exposure.

- Systemic effects are limited to findings in the bladder in mice at 0.27 mg/L/6hr/day at 6-months, 0.27 mg/L/6hr/day at 1-year and 0.09 and 0.27 mg/L/6hr/day at 2-years. This concentration is below the STOT RE Cat 2 cut-off at 6-months (0.5 mg/L) and at 2-years (0.125 mg/L). However as these studies used whole-body exposure this is likely to be an underestimate of actual exposure as some oral exposure is likely to have occurred as well. It is known that in the mouse the bladder is a target organ following oral exposure (Anon. 52 (1997)) and additional oral exposure in the inhalation study may have contributed to systemic toxicity, vis a vis, bladder effects, which cannot be solely or definitely attributed to inhalation exposure.

- Systemic effects are limited to a single species (mouse) and there are no adverse systemic effects in rats following repeated-dose inhalation exposure.

10.13 Aspiration hazard

Not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 83: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready biodegradability			
OECD 301D, July 17, 1992 GLP Test substance, Telone II, TSN 102401, 98.7% purity, 51/49 cis/trans ratio	% degradation 1,3-dichloropropene 7 days: 0.9% 14 days: 1.4% 21 days: 2.2% 28 days: 4.9%	OECD 301D Closed-Bottle Test Incubation for 28 days % degradation results based on Biological Oxygen Demand (BOD) Guideline threshold for ready biodegradability and 14-day window were not met; **Conclusion**: 1,3-D is ***not*** "readily biodegradable"	Anonymous 74, 2002
Hydrolysis			
OECD 111; EC Directive 84/449/EEC Annex V GLP Test material: pure grade cis 1,3-D; 98.1%	DT50 (**hours**) cis 1,3-dichloropropene @ 50°C pH 4: 7.15 pH 7: 6.17 pH 9: 5.27 @ 60°C pH 4: 2.45 pH 7: 2.38 pH 9: 2.15 @ 70°C pH 4: 1.12 pH 7: 1.32 pH 9: 1.30 **Arrhenius at 25ºC (calculated)** pH 4: 100 pH 7: 64.5 pH 9: 37.6	Investigated at pH 4, 7 and 9 incubated in the dark at 50, 60 and 70°C for 5 days in sterile buffered solutions **Conclusion**: Cis 1,3-D is rapidly degraded by hydrolysis to form principally the cis 3-chloroallyl alcohol	Anonymous 75, 1990
OECD 111; EC Directive	DT50 (**hours**) trans 1,3-dichloropropene @ 50°C	Test material: pure grade trans1,3-D Investigated at pH 4, 7 and 9	Anonymous 76, 1998

84/449/EEC Annex V GLP Test material: pure grade trans 1,3-D; 97.8%	pH 4: 4.59 pH 7: 4.85 pH 9: 4.39 @ 25°C pH 4: 118 pH 7: 114 pH 9: 114 **Arrhenius at 20ºC (calculated)** pH 4: 226 pH 7: 215 pH 9: 221	incubated in the dark at 25 and 50°C for 5 days in sterile buffered solutions **Conclusion**: Trans 1,3-D is rapidly degraded by hydrolysis to form principally the Trans 3-chloroallyl alcohol	
US EPA 161-1, EEC Method C7 and OECD Method 111 GLP Test material: Metabolite ^{14}C-3-Chloroallyl Alcohol, radiochemical purity 97.9%	3-Chloroallyl alcohol is hydrolytically stable at pH 5, 7 and 9.	No significant degradation of 3-Chloroallyl alcohol was observed in a preliminary experiment at pH 5, 7 and 9 at 50°C over 5 days or in the definitive experiment conducted at 25°C over a 30 day period.	Anonymous 77, 1999
US EPA 161-1, EEC Method C7 and OECD Method 111 GLP Test material: Metabolite ^{14}C-3-Chloroacrylic acid, radiochemical purity 97.6%	3-Chloroacrylic acid is hydrolytically stable at pH 5, 7 and 9.	No significant degradation of 3-Chloroacrylic acid was observed in a preliminary experiment at pH 5, 7 and 9 at 50°C over 5 days or in the definitive experiment conducted at 25°C over a 30 day period.	Anonymous 78, 1999
US EPA 161-1, EEC Method C7 and OECD Method 111 GLP Test material: Metabolite ^{14}C-3-Chloroacrylic acid radiochemical purity 92.4%	3-Chloroacrylic acid is hydrolytically stable in natural water	Hydrolysis of [14C]3-Chloroacrylic acid was studied in the dark at 50 °C in two natural water systems for 5 days.	Anonymous 79, 2014
Water, water-sediment and soil degradation data			

SETAC-Europe guideline, Procedures for Assessing the Environmental Fate and Ecotoxicology of Pesticides, Part 1, Section 8.2 (1995), with modification on temperature GLP Test material ^{14}C cis/trans 1,3-Dichloropropene, radiochemical purity >99%	<u>DT50 (**days**) 1,3-dichloropropene @ 25°C</u> 1,3-D first order pH 7.4: 4.9 1st order, non linear, ModelMaker pH 7.4: 4.6 Two compartment order, non linear, ModelMaker pH 7.4: 4.5 <u>Metabolites @ 25°C</u> Two compartment order, non linear, ModelMaker 3-Chloroallyl alcohol pH 7.4: 0.3 days 3-Chloroacrylic acid pH 7.4: 7.2 days	Route and rate of aquatic degradation was investigated in a representative water/sediment system (of US origin) under aerobic laboratory conditions at 25ºC Application was made to the aqueous layer to mimic introduction into aquatic systems via run off into an open body of water Test system consisted of a loamy sand sediment and associated natural water (pH 7.4) The dissipation of 1,3-D from the water/sediment system was found to correlate well to first-order kinetics **Conclusion**: 1,3-D rapidly dissipates with a DT50 of 4.5-4.9 days. . The major dissipation processes under these conditions are the formation of 3-chloroallyl alcohol (up to 6.2% AR), 3-chloroacrylic acid (up to 9.5% AR) and CO_2 loss (up to 42.5% AR). There is no accumulation of either parent or metabolites in the sediment or water phases.	Anonymous 80, 1999
SETAC-Europe guideline, Procedures for Assessing the Environmental Fate and Ecotoxicology of Pesticides, Part 1, Section 1.1 (1995) GLP Test material: ^{14}C cis/trans 1,3-Dichloropropene, radiochemical purity 98.6%	1,3-D was rapidly dissipated in soil under aerobic conditions. The DT50(lab) values ranged between 8.8 to 15.5 days at 20°C, with degradation tending to be fastest in soils with high organic carbon. Dissipation rate includes microbial degradation and two other significant degradation routes for 1,3-D, degradation due to hydrolysis and volatilisation. Degradation 1,3-D in soil under aerobic conditions was rapid. The DT50(lab) values ranged between 11.7 to 27.1 days at 20°C (arithmetic mean 16.6 days), with degradation tending to be fastest in soils with high organic carbon. Degradation of 3-chloroallyl alcohol in soil was so rapid that it could only be estimated with DT50(lab) of 0.2 day (arithmetic mean). Degradation of 3-chloroacrylic acid in soil correlated well to non-linear kinetics with DT50(lab) values ranged between 6.0 to 19.8 days at 20°C (arithmetic mean 13.6 days	Aerobic degradation of 14C 1,3-D was investigated in four representative European agricultural soil types under laboratory conditions at 20°C and 40% moisture holding capacity (MHC) in the dark as well as 10°C and 40% MHC and 20°C and 20% MHC for up to 120 days **Conclusion**: The degradation and dissipation of 1,3-D in viable aerobic soil demonstrated that both biotic and abiotic processes are significant. 1,3-D is rapidly degraded and dissipated under aerobic conditions Microbial degradation, hydrolysis and volatilisation are all major routes of dissipation Degradation of alcohol metabolite to the acid metabolite is rapid (DT_{50}, 0.2d). The acid metabolite soil DT_{50} ranged between 6 to 19.8 days at 20°C.	Anonymous 81, 2002

OECD 111 USEPA OCSPP 835.2120 EU Guideline SANCO/3029/99 rev. 4 GLP Test material: cis 1,3-dichloropropene, TSN 100275, Purity 98.9%	DT50 (**days**) cis 1,3-chloropropene @ 20°C HPLC Water pH 7: 7.0d Natural Water pH 7: 11.1d	Evaluated in sterilized buffered HPLC grade water at pH 7 and unsterilized natural water buffered at pH 7 Conducted for three days at 20°C ± 0.5°C for durations ranging from of 24 hours to 31 days	Anonymous 82, 2014
Photochemical degradation			
US EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 161-2 (1982) Test material: ^{14}C cis/trans 1,3-Dichloropropene, radiochemical purity 99%	DT50 (**days**) 1,3-chloropropene @ 25°C Exposed to xenon light pH 7: 5.7 Dark controls pH 7: 5.8 Photolysis pH 7: 650	Investigated in sterile aqueous buffer (pH 7) under artificial sunlight (xenon) at 25°C over 16 days **Conclusion**: Rapid hydrolysis of 1,3-dichloropropene was observed, thus photolysis is not a significant route of environmental degradation (calculated photolysis DT_{50} of 650 days)	Anonymous 83, 1996

11.1.1 Ready biodegradability

One test is available to assess the ready biodegradability of 1,3-dichloropropene (Telone II, Anonymous 74, 2002). 1,3-Dichloropropene was tested for ready biodegradability according to OECD 301D Closed-Bottle-Test. Under the chosen conditions, degradation equal to 5% of the calculated biological demand was measured after 28d. The threshold for ready biodegradability as well as the 14-days window, as required by the OECD guideline 301D were not met. Therefore the test item must be considered not readily biodegradable.

11.1.2 BOD_5/COD

No studies that investigated the BOD5/COD of 1,3-dichloropropene were available for review.

11.1.3 Hydrolysis

The hydrolytic degradation of cis and trans 1,3-dichloropropene was investigated in two studies, Cis-isomer (Anonymous 75, 1990), Trans-isomer (Anonymous 76, 1998). These studies were conducted under GLP standards according to OECD 111 (Hydrolysis as a function of pH). Tests were conducted in sterile, buffered solutions at three pHs (4, 7, and 9). For cis-1,3-dichloropropene a single first order DT50 at 25°C of 100, 54.5, and 38 hours was obtained at pH4, pH7, and pH9 respectively. For trans-1,3-dichloropropene a single first order DT50 at 20°C of 224, 215, and 221 hours was obtained at pH4, pH7, and pH9 respectively. In both studies the degradation appeared to be independent of pH. The degradation reaction in both studies appears to proceed by a S_N1 type mechanism via a resonance stabilised carbonium ion to form the corresponding 3-chloroallyl alcohol (cis/trans 3-chloro-1-hydroxypropene). Based on these two studies, it can be concluded that 1,3-dichloropropene is rapidly degraded by hydrolysis.

The hydrolysis of ^{14}C-3-chloroallyl alcohol, specific activity 1.91 mCi/mmol (batch INV 1444, radiochemical purity 97.9%) was investigated at pH 5, 7 and 9 (Anonymous 77, 1999). No significant degradation of

3-chloroallyl alcohol was observed in a preliminary experiment at pH 5, 7 and 9 at 50°C over 5 days or in the definitive experiment conducted at 25°C over a 30 day period which confirmed the hydrolytic stability.

The hydrolysis of ^{14}C-3-chloroacrylic acid, specific activity 1.93 mCi/mmol (batch INV 1463, radiochemical purity 97.6%) was investigated at pH 5, 7 and 9 (Anonymous 78, 1999). No significant degradation of 3-chloroacrylic acid was observed in a preliminary experiment at pH 5, 7 and 9 at 50°C over 5 days or in the definitive experiment conducted at 25°C over a 30 day period which confirmed the hydrolytic stability. The hydrolysis of ^{14}C-3-chloroacrylic acid, (TSN 101702, radiochemical purity 92.4%) was investigated in 2 sources of Natural water, stored in the dark at 50°C over 5 days (Anonymous 79, 2014). No significant degradation of 3-chloroacrylic acid was observed.

11.1.4 Other convincing evidence

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

No field studies or monitoring data that are relevant to the Classification and Labeling of 1,3-dichloropropene are reported here.

11.1.4.2 Inherent and enhanced ready biodegradability tests

1,3-D is considered as not readily biodegradable and the aerobic mineralization in surface water was not investigated further. As a result, no studies that investigated the inherent or enhanced ready biodegradability of 1,3-dichloropropene were available for review.

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

One study was available that investigated the degradation of 1,3-dichloropropene in unsterilized natural water at pH 7 (Anonymous 82, 2014). This study was conducted under GLP standards according to internationally accepted guidelines (e.g., OECD and USEPA). Incubation times ranged from 24 hours to 31 days. A concurrent evaluation was done in sterile HPLC water at pH 7. DT50 values of 11.1 and 7.0 days were determined for the natural and HPLC waters, respectively.

A study was available that investigated the degradation of 1,3-dichloropropene in water-sediment systems, which would mimic natural surface water scenarios (Anonymous 80, 1999). The test material (^{14}C cis/trans 1,3-Dichloropropene, Inv No 1472) had a radiochemical purity of >99%. This study was conducted under GLP standards according to SETAC Europe guideline Part 1 Section 8.2 (1995). Application was made to the aqueous layer to mimic introduction into aquatic systems via run off or drift into an open body of water. The test system consisted of a loamy sand sediment and associated natural water (pH 7.4). When applied to the water phase to mimic introduction into aquatic systems via run off into an open body of water, 1,3-D rapidly dissipates with a DT50 of 4.5-4.9 days. The major dissipation processes under these conditions are the formation of 3-chloroallyl alcohol (up to 6.2% AR), 3-chloroacrylic acid (up to 9.5% AR) and CO_2 loss (up to 42.5% AR). No major metabolites >10% AR are formed. There is no accumulation of either parent or metabolites in the sediment or water phases.

A study was also available that investigated the aerobic degradation of 1,3-dichloropropene in four representative European agricultural soils (Anonymous 81, 2002). The test material (^{14}C cis/trans 1,3-Dichloropropene, Inv No 1550) had a radiochemical purity of 98.6%. This study was conducted under GLP standards according to SETAC Europe guideline Part 1 Section 1.1 (1995). Incubations were conducted for up to 120 days. The DT50(lab) values ranged between 8.8 to 15.5 days at 20°C, with degradation tending to be fastest in soils with high organic carbon. 1,3-D was rapidly degraded and dissipated under aerobic conditions and microbial degradation, hydrolysis and volatilisation were all major routes of dissipation. Degradation of 3-chloroallyl alcohol in soil was so rapid that it could only be estimated with DT50(lab) of 0.2 day (arithmetic mean). Degradation of 3-chloroacrylic acid in soil correlated well to non-linear kinetics with DT50(lab) values ranged between 6.0 to 19.8 days at 20°C (arithmetic mean 13.6 days). There was not a significant difference in the soil dissipation or degradation rates between *cis* and *trans* isomers of 1,3-D. The *trans* isomer of 3-chloroacrylic acid was found be faster degrading than the *cis* isomer.

11.1.4.4 Photochemical degradation

One test was available to assess potential for photochemical degradation of 1,3-dichloropropene (Telone II, Anonymous 83, 1996). This study were conducted under GLP standards according to EPA guidelines (Subdivision N, Paragraph 161-2 (1982)). In this study, the rate of 1,3-D breakdown in illuminated samples from sterile aqueous solutions was comparable to that which occurred by hydrolysis in the dark controls. This confirms the predominant route of 1,3-D degradation in water will be via hydrolysis and not photolysis. Thus, it can be concluded that photolysis is not a significant pathway for the environmental degradation of 1,3-D.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for 1,3-dichloropropene.

11.2.1 Summary of data/information on environmental transformation

11.3 Environmental fate and other relevant information

No additional data is presented for the purposes of Classification and Labeling.

11.4 Bioaccumulation

The summary of partition coefficient test data evaluated during Annex I inclusion of 1,3-dichloropropene and submitted for the purposes of EU renewal is reported below. Only information considered adequate, reliable and relevant for the classification proposal has been included.

Table 84: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
EEC A.8 Partition coefficient n-octanol/water GLP	cis-isomer: 2634 (98.1%) Log Kow: 1.82 at 20°C	Purified water (Not pH dependant) The study is acceptable	Anonymous 75, 1990
EEC A.8 Partition coefficient n-octanol/water GLP	trans-isomer: TSN100276 (97.8%) Log Kow: trans-isomer: 2.1 at 20°C	Purified water (Not pH dependant) The study is acceptable	Anonymous 84, 1998
OECD Method 117 and EPA/OPPTS·830.7570. Partition coefficient n-octanol/water GLP	cis-3-chloroallyl alcohol metabolite: AGR 164303 (96.1%) Log Kow: 0.78 at 25°C	HPLC Procedure The study is acceptable	Anonymous 85, 2002
OECD Method 117 and EPA/OPPTS·830.7570. Partition coefficient n-octanol/water GLP	trans-3-chloroallyl alcohol metabolite: AGR 159855 (96.9%) Log Kow: 0.72 at 25°C	HPLC Procedure The study is acceptable	Anonymous 85, 2002

OECD Method 117 and EPA/OPPTS·830.7570. Partition coefficient n-octanol/water GLP	cis-3-chloroacrylic acid metabolite: TSN 101343 (>99%) Log Kow: <0.30 at 20°C	HPLC Procedure (Not pH dependant) The study is acceptable	Anonymous 86, 2002
OECD Method 117 and EPA/OPPTS·830.7570. Partition coefficient n-octanol/water GLP	trans-3-chloroacrylic acid metabolite: TSN 101371 (>99%) Log Kow: <0.30 at 20°C	HPLC Procedure (Not pH dependant) The study is acceptable	Anonymous 86, 2002

11.4.1 Estimated bioaccumulation

The log Kow for cis- 1,3-dichloropropene is 1.82 at 20°C (Anonymous 75, 1990) and for trans- 1,3-dichloropropene is 2.1 at 20°C (Anonymous 84, 1998). The log Kow for the cis-alcohol and cis-acid metabolites are 0.78 and <0.3 respectively at 20°C (Anonymous 85, 2002 and Anonymous 86, 2002). The log Kow for the trans- alcohol and trans-acid metabolites are 0.72 and <0.3 respectively at 20°C. (Anonymous 85, 2002 and Anonymous 86, 2002). The log Kow threshold criteria for classification as potential to bioaccumulate, according to CLP regulation section 4.1.2.8, is 4.

Based on the measured log Kow values for 1,3-D and the rapid hydrolytic degradation demonstrated in water, bioconcentration and subsequent bioaccumulation through the food chain is not expected.

11.4.2 Measured partition coefficient and bioaccumulation test data

Partition coefficients octanol/water for 1,3-dichloropropene was determined by the Shake Flask Method according to the EEC A.8 giving the results as follow: cis-1,3-dichloropropene is 1.82 at 20°C and for the trans-1,3-dichloropropene is 2.1 at 20°C. The Kow for the two principle soil and water metabolites indicated that they were not likely to bioaccumulate.

The studies are considered valid and reliable. They are relevant for classification purposes.

11.5 Acute aquatic hazard

The summary of the aquatic toxicity studies evaluated during Annex I inclusion of 1,3-dichloropropene is reported below. Only information considered adequate, reliable and relevant for the classification proposal has been included.

The available acute toxicity data for relevant metabolites of 1,3-dichloropropene (3 chloroprop-2-en-1 ol (3-CAA) and 3-chloroacrylic acid (3-CACA)) revealed toxicity values similar to the parent substance. Therefore, the studies with these metabolites are not described here in detail.

11.5.1 Acute (short-term) toxicity to fish

Table 85: Summary of relevant information on acute toxicity to fish

Method	Species	Test material	Results[1]	Remarks	Reference
Fish					
EC Directive 92/69/EEC. Guideline C1; OECD 203; US EPA 72-1)	Rainbow trout, *Oncorhynchus mykiss* Walbaum	Telone II (1,3-Dichloropropene, 100%)	96-h LC50: 2.78 mg as/L (95% CI not calculable), based on mean measured concentrations	96-hour flow-through exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met**	Anonymous 91, 2001 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.1/1
US EPA OPPTS 850.1075; ASTM Standard E729-88a	Sheepshead minnow, *Cyprinodon variegatus*	Telone II (1,3-Dichloropropene, 96%)	96-h LC50: 0.87 mg as/L (95% CI 0.57-1.1), based on mean measured concentrations	96-hour flow-through exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met**	Anonymous 92, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.1/2
EC Directive 92/69/EEC. Guideline C1, US EPA. 72-1.	*Oncorhynchus mykiss*	Metabolite 3 chloroprop-2-en-1 ol; Chloroallyl Alcohol (3-CAA)	96-hour LC50 = 0.986 mg 3-CAA/L (based on mean measured concentrations)	96 hour static renewal exposure Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met**	Anonymous 93, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.1/3
EC Directive 92/69/EEC. Guideline C1, US EPA. 72-1.	*Oncorhynchus mykiss*	Metabolite 3-chloroacrylic acid (3-CACA)	96-hour LC50 = 69.5 mg 3-CACA/L(based on mean measured concentrations)	96 hour static exposure Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met**	Anonymous 94, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.1/4

[1] Indicate if the results are based on the measured or on the nominal concentration.

The below summary covers fish, aquatic invertebrate testing of (Z)-1,3-dichloropropene (Cis isomer):

Anonymous 87 (1989)

Title	Cis-1,3-Dichloropropene: Acute toxicity to *Salmo gairdneri, Daphnia magna* and *Selenastrum capricornutum*
Guidelines	Official Journal of the European Communities No. L251 Part C: Methods for the Determination of Ecotoxicity. C1 Acute Toxicity for Fish; C2 Acute toxicity for Daphnia. Federal Register Part II, Environmental Protection Agency, 40 CFR Parts 796, 797 and 798. Toxic Substances Control Act Test Guidelines; Final Rules.
GLP	Yes

METHODOLOGY

Cis-1,3-Dichloropropene Batch No.1986; 88009. Purity: Cis-1,3-D content 95.45%.

The acute toxicity of Cis-1,3-Dichloropropene to three aquatic species was determined for the durations and test conditions outlined below.

Rainbow trout (*Salmo gairdneri*); LC_{50} determined at 6, 24, 48, 72 and 96h; semi-static

Crustacean (*Daphnia magna*); EC_{50} determined at 6, 12, 24, and 48h; static

Algae (*Selenastrum capricornutum*); E_bC_{50} determined at 48, and 72h; E_rC_{50} determined at 24-48h, and 24-72h; static (sealed)

Test material concentrations were measured during the study, all quoted concentrations are mean measured values.

Salmo gairdneri

A 96 h semi-static toxicity test was carried out with daily renewal of the test solutions. On day 1 of the test seven, 20 litre glass round bottom reaction vessels were each filled with filtered dechlorinated mains water. Quantities of a stock solution of cis-1,3-dichloropropene in filtered dechlorinated mains water were added to six of these to give an approximately logarithmic series of nominal concentrations between 2.9 and 49 mg/L. The seventh vessel received no cis-1,3-dichloropropene and served as a control. Ten *S, gairdneri* were placed in each vessel and the vessels topped up with test medium and sealed with a glass plate to exclude air.

On day 2 of the test a further four vessels were prepared at nominal concentrations between 0.27 and 1.6 mg/L. This was deemed necessary because it was considered likely that toxic effects would extend to the lowest concentration in the original concentration series.

At intervals after the start of the test (3, 6, 24, 48, 72 and 96 h) the fish were observed and the number of fish exhibiting toxic symptoms was recorded. The symptoms were classified into one of five categories from (a) no toxic symptoms to (e) dead (see Table 106). After completing these observations at 24, 48, 72 and 96 h any dead fish were removed, dissolved oxygen concentration and pH were measured, the total hardness of the water was determined and the test solutions were renewed (not at 96 h).

The temperatures of the solutions during the test was 13-17°C, pH was 7.0- 7.8, the total hardness of the water was 226-258 mg/L as $CaC0_3$ and the concentration of dissolved oxygen was 6.4-10.2 mg/L.

The 6, 24, 48, 72 and 96 h LC50 values (those concentrations calculated to cause a 50% mortality after 6, 24, 48, 72 and 96 h exposure) were calculated using the moving average-angle method (US Environmental Protection Agency, 1985) after log transformation of the concentrations.

Daphnia magna

A 48 h toxicity test was carried out without renewal of the test solutions. Quantities of a stock solution of cis-1,3-dichloropropene in reconstituted fresh water were added to quadruplicate sets of 150 ml conical flasks containing reconstituted fresh water to give, when completely full, an approximately logarithmic series of nominal concentrations ranging from 0.58-36 mg/L. Four flasks served as controls and received no cis-1,3-

dichloropropene. Five *D. magna*, less than 24 h old, were allocated to each flask and the flasks sealed with a glass cover slip. After 3, 6, 12, 24 and 48 h the numbers of immobilized *D. magna* were recorded. *D. magna* were considered to be immobile if, when they were observed through the base of the flask they did not swim during a 15 second period of observation. During the test the temperature of the test solutions was 18-22°C, pH was 7.6-8.4, the total hardness of the water was 176 mg/L as $CaC0_3$ and the concentration of dissolved oxygen was 8.8-9.8 mg/L.

The 6, 12 and 48 h EC50 values (those concentrations calculated to cause a 50% immobilization after 6, 12 and 48 h exposure) were calculated using the moving average-angle method (US Environmental Protection Agency, 1985). The 24 h EC50 value was calculated using probit analysis (Finney, 1971). All calculations were performed after log transformation of the concentrations.

Selenastrum capricornutum

A 72 h growth experiment was carried out. Quantities of a stock solution of cis-1,3-dichloropropene prepared in algal growth media were added to triplicate sets of 300 ml full volume conical flasks containing algal growth media, so that when completely full an approximately logarithmic series of nominal concentrations ranging from 2.2-100 mg/L was produced. A further six flasks were filled with algal growth medium only to serve as controls. Each flask was inoculated with sufficient *S. capricornutum* cells to give an initial concentration of 103 cells ml^{-1}. Two autoclaved marbles were placed in each flask (to stir the contents of the flask during incubation) and the flasks sealed with a ground-glass stopper. The flasks were incubated in a cooled orbital incubator (100 cycles min^{-1}) under constant illumination (-3000 lux) at 25-28°C for 72 h. At 24 h intervals after the start of incubation cell counts were made using a Coulter Counter. The pH of the test solutions during the test was 7.3-7.6.

Effects were evaluated using two approaches:

(a) Comparison of areas under the growth curves.

(b) Comparison of average specific growth rates.

FINDINGS

Test material concentrations were measured during the study, all quoted concentrations are mean measured values.

Salmo gairdneri

The results of the toxicity test with *S. gairdneri* are given in the following table.

Table 86: Symptoms and mortality observations

Mean measured concentration (mg/L)	Number of fish	Observation time and symptom classification																													
		3 h					6 h					24 h					48 h					72 h					96 h				
		a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
0 (control)	10	10					10					10					10					10					10				
0.19	10	10					10					10					10					10					10				
0.37	10	10					10					10					10					10					10				
0.59	10	10					10					10					10					10					10				
1.1	10	10					10					10					10					10					7	1	2		

2.1	10	10					10					6	4						2	7	1				10				10
4.1	10	10					10							6	3	1					10				10				10
6.7	10	10					10									10					10				10				10
12	10	10						2	3	4	1					10					10				10				10
26	10		6	3	1					9	1					10					10				10				10
37	10				10						10					10					10				10				10

a) Number of fish exhibiting no toxic symptoms
b) Number of fish swimming normally but exhibiting toxic symptoms (e.g. increased cough frequency or hyperventilation)
c) Number of fish swimming abnormally (e.g. on side or back)
d) Number of fish immobilised (e.g. lying on bottom of tank or floating at surface, but still alive)
e) Number of fish dead

The highest mean measured concentration of cis-1,3-dichloropropene causing no mortality within the period of the test was 0.59 mg/L. The lowest concentration causing 100% mortality within the period of the test was 2.1 mg/L.

Other sublethal toxic effects were only recorded during the period of the test at mean measured concentrations ≥1.1 mg/L.

The 6, 24, 48, 72 and 96 h LC_{50} values were calculated to be 27, 4.4, 2.4, 1.6 and 1.6 mg/L respectively (95% confidence limits 25-30; 3.9-5.1, 2.1-2.9; 1.4-1.8 and 1.4-1.8 mg/L). The slope of the concentration/percentage response curve could not be determined using the data obtained in the test.

Table 87: Results

Result	**mg/L, measured (95% confidence limits)**
	cis-1,3-dichloropropene
6h LC_{50}	27 (25-30)
24h LC_{50}	4.4 (3.9-5.1)
48h LC_{50}	2.4 (2.1-2.9)
72h LC_{50}	1.6 (1.4-1.8)
96h LC_{50}	1.6 (1.4-1.8)

Daphnia magna

The results of the toxicity test with *D. magna* are given in the following table.

Table 88: Immobilisation observations

Mean measured concentration (mg/L)	Number of *Daphnia Magna*	Number immobilised				
		3 h	6 h	12 h	24 h	48 h
0 (control)	5	0	0	0	0	0
	5	0	0	0	0	0
	5	0	0	0	0	0
	5	0	0	0	0	0
0.32	5	0	0	0	0	0
	5	0	0	0	0	0
	5	0	0	0	0	0
	5	0	0	0	0	0
0.55	5	0	0	0	0	0
	5	0	0	0	0	0

	5	0	0	0	0	0
	5	0	0	0	0	0
1.1	5	0	0	0	0	0
	5	0	0	0	0	0
	5	0	0	0	0	0
	5	0	0	0	0	0
1.4	5	0	0	0	0	2
	5	0	0	0	0	2
	5	0	0	0	1	1
	5	0	0	0	0	3
2.9	5	0	0	0	3	5
	5	0	0	0	1	5
	5	0	0	1	3	5
	5	0	0	0	4	5
5.0	5	0	0	5	5	5
	5	0	0	5	5	5
	5	0	0	5	5	5
	5	0	0	5	5	5
9.4	5	0	1	5	5	5
	5	0	0	5	5	5
	5	0	0	5	5	5
	5	0	0	5	5	5
16.4	5	0	5	5	5	5
	5	0	5	5	5	5
	5	0	5	5	5	5
	5	0	5	5	5	5

The highest mean measured concentration of cis-1,3,-dichloropropene causing no immobilization within the period of the test was 1.1 mg/L. The lowest concentration causing 100% immobilization within the period of the test was 2.9 mg/L.

The 6, 12 24 and 48 h EC_{50} values were calculated to be 11; 3.3; 2.6 and 1.4 mg/L respectively (95% confidence/fiducial* limits 9.8-12; 3.0-3.6; 2.2-3.1* and 1.3-1.7 mg/L). The slope of the concentration/percentage response curve could not be determined using the data obtained in the test.

Table 89: Results

Result	**mg/L, measured (95% confidence limits)**
	cis-1,3-dichloropropene
6h EC_{50}	11 (9.8-12)
12h EC_{50}	3.3 (3.0-3.6)
24h EC_{50}	2.6 (2.3-3.1*)
48h EC_{50}	1.4 (1.3-1.7)

* fiducial limit

Selenastrum capricornutum

The results of the toxicity test with *S. capricornutum* are given in the following table.

Table 90: Cell concentration data

Mean measured concentration (mg/L)	Cell concentration (cells/mL x10^6)	Mean cell concentration (cells/mL x10^6)		Calculated mean area under the growth curve (A)	Reduction in 'A' relative to controls (IA%)	Calculated specific growth rate (μ)	Reduction in μ relative to controls (%)

	24 h	48 h	72 h	24 h	48 h	72 h		0-24h	0-48h	0-72h	0-24h	0-48h	0-72h	t24-t48	t24-t72	t24-t48	t24-t72
Control (0.09)	0.0052	0.056	0.21	0.0045	0.041	0.22		41800	563600	3711600	-	-	-	0.094	0.082	-	-
	0.0038	0.042	0.19														
	0.0072	0.033	0.18														
	0.0038	0.040	0.23														
	0.0038	0.034	0.33														
	0.0031	0.041	0.20														
0.82	0.0035	0.044	0.35	0.0035	0.043	0.26		30400	568800	4224800	27	0	0	0.11	0.089	0	0
	0.0036	0.045	0.23														
	0.0035	0.041	0.21														
1.8	0.0049	0.035	0.19	0.0048	0.031	0.19		45600	447200	3111200	0	21	16	0.077	0.077	18	6
	0.0050	0.031	0.20														
	0.0045	0.026	0.19														
3.9	0.0042	0.015	0.017	0.0042	0.016	0.014		38000	25200	588000	9	55	84	0.055	0.025	41	69
	0.0046	0.016	0.011														
	0.0037	0.016	0.015														
8.9	0.0017	0.0045	0.0026	0.0022	0.0038	0.0025		14400	62000	113200	66	89	97	0.023	0.007	75	91
	0.0022	0.0038	0.0001														
	0.0027	0.0030	0.0048														
25	0.0026	0.0021	0	0.0031	0.0027	0		24800	70000	0	41	88	100	0.003	0	97	100
	0.0037	0.0018	0														
	0.0029	0.0042	0														
60	0.0030	0	0	0.0029	0	0		23200	0	0	44	100	100	0	0	100	100
	0.0024	0	0														
	0.0034	0	0														

The 48 and 72 h E_bC_{50} values calculated on the basis of the areas under the growth curves were 4.1 and 2.8 mg/L respectively. A 24 h E_bC_{50} value could not be reliably calculated from the results of the study. 95% fiducial limits for the 48 and 72 h E_bC_{50} values were 2.0-8.3 and 2.2-3.5 mg/L respectively.

The E_rC_{50} (24-48 h) and E_rC_{50} (24-72 h) values, determined by analysis of average specific growth rates were 4.6 and 3.1 mg/L respectively.

The no observed effect concentration (NOEC) determined by analysis of both the areas under the growth curves and average specific growth rates was 0.82 mg/L.

Table 91: Results

Result	**mg/L, measured (95% confidence limits)**
	cis-1,3-dichloropropene
48 h E_bC_{50}	4.1 (2.0-8.3)
72 h E_bC_{50}	2.8 (2.2-3.5)
E_rC_{50} (24-48 h)	4.6
E_rC_{50} (24-72 h)	3.1
NOEC	0.82

CONCLUSIONS

Salmo gairdneri

The 96 h LC_{50} value for Cis-1,3-Dichloropropene was calculated to be 1.6 mg/L (95% confidence limits 1.4-1.8 mg/L).

The validity criteria laid out in OECD guideline 203 are met, it should be noted oxygen concentrations were measured in mg/L as opposed to % saturation however measurements at the top concentration (8.4-10.2mg/L) lay within the range observed in the control samples (6.4-10.2mg/L).

Daphnia magna

The 48 h EC_{50} value for Cis-1,3-Dichloropropene was calculated to be 1.4 mg/L (95% confidence limits 1.3-1.7 mg/L). The validity criteria laid out in OECD guideline 202 are met.

Selenastrum capricornutum

According to the ECHA Chapter R.7b: Endpoint specific guidance "*Often both acute growth rate EC50 (ErC50) and biomass (EbC50) endpoints are reported however the latter should not be used*.". The 24-72h E_rC_{50} of 3.1 mg/L is therefore the selected acute endpoint for *Selenastrum capricornutum*, with a NOEC of 0.82 mg/L exposed to for Cis-1,3-Dichloropropene. The information available in the report does not allow for a full comparison with the OECD 201 validity criteria as cell counts were not conducted at study initiation. The mean cell concentration increased from 0.0045 x 10^6 cells/mL at 24 hours to 0.22 x 10^6 cells/mL at 72 hours, which is a factor of 48 increase indicating exponential growth.

The below summary summarises the acute toxicity of (Z)-1,3-dichloropropene (Cis isomer) and (E)-1,3-dichloropropene (Trans isomer) to the sheepshead minnow, *Cyprinodon variegatus*. It is not considered reliable for the (Z)-1,3-dichloropropene (Cis isomer) and only considered reliable with restriction for (E)-1,3-dichloropropene (Trans isomer).

Anonymous 88 (1978)

Title	The Acute Toxicity of Cis and Trans 1,3 Dichloropropene to the sheepshead minnow, *Cyprinodon variegatus*.
Guidelines	Committee on Methods for Toxicity Tests with Aquatic Organisms. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Ecological Research Series , EPA 660/3-75-009. Standard Methods for the examination of Water and Wastewater. 13th Edition . Washington, D.C.: American Health Association American Water Works Association, and Water Pollution Control Federation, 1971.
GLP	Not GLP

METHODOLOGY

Cis and trans 1,3 Dichloropropene were evaluated for acute toxicity to the sheepshead minnow, *Cyprinodon variegatus* using a continuous flow test method. The compounds tested were Dow production samples.

The test fish were collected from the Surfside Beach salt marsh area.

Collected specimens were kept in the laboratory for at least 2 weeks prior to testing where the water temperature was maintained between 81-82° F. The photoperiod was set at 18 Light:6 Dark.

Stock solutions of cis and trans 1, 3-dichloropropene were made by mixing the sample to be tested in 50 gallons of seawater. The solution was then stirred vigorously so that complete dissolution was achieved. The 50-gallon stock solution was then allowed to equilibrate and mixing was then continued at a slow rate. The test substances were then pumped to a proportional dilutor, Mount and Brungs (1967).

A standard 96-hour LC50 was determined according to the test methods described by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Five fish were exposed at each concentration in the preliminary screening, while 20 individuals per concentration were used in the full scale tests. Samples

were collected and verified every 24 hours by Gas Chromatography using an Electron Capture detector. The column used for the trans consisted of a 6' x 1/8" glass column OV-101 on chromosorb W-HP. Cis determination was also done by Electron Capture using a 6' x 1/4" glass column with 10% OV-101 on chromosorb H.

Chemical characteristics of the water, analyzed according to Standard Methods (APHA, 1971) were : pH 7.9; 15,620 ppm chloride (C1-); and 5.8 - 6.0 ppm Dissolved Oxygen.

Results are reported in terms of the concentration necessary to produce death to 50% of the individuals (LC_{50}) within the 96-hour exposure period. Log probit paper was used to calculate percent mortality versus concentration.

FINDINGS

Cis and Trans 1,3 Dichloropropene were tested for their acute toxicity to the sheepshead minnow, *Cyprinodon variegatus*. Flowthrough testing involved the use of a proportional dilutor , Mount and Brungs (1967). The LC_{50} for Cis and trans 1,3 Dichloropropene is 0.068 ppm and 0.290 ppm, respectively.

CONCLUSIONS

Cis and Trans 1,3 Dichloropropene were tested for their acute toxicity to the sheepshead minnow, *Cyprinodon variegatus*. Flowthrough testing involved the use of a proportional dilutor, Mount and Brungs (1967). The LC_{50} for Cis and trans 1,3 Dichloropropene is 0.068 ppm and 0.290 ppm, respectively. The test concentrations were verified throughout the 96 hour exposure duration and the minimum number of fish per concentration were aligned with OECD 203 guidance.

However, there are a number of deficiencies in the experimental work or the reporting as the study was not conducted under GLP. Details on controls and dissolved oxygen levels as a percentage of full saturation are not included in the report; it is therefore not possible to conclude if the study meets all of the validity criteria specified in OECD 203. Further deficiencies in the treatment concentration selection, statistical analysis and reporting are discussed below for each of the isomers.

(Z)-1,3-dichloropropene (Cis isomer): Only 3 test concentrations were plotted (not 5 required in both the 1975 guidance cited and OECD 203). Of the three data points one showed 0% mortality, one showed 100% mortality and one data point where partial mortality was observed. The results derived from the plot of these three data points on probit paper cannot be considered reliable, per OECD 203 “experiment results in only one concentration with partial mortality or no concentration with partial mortality”.

(E)-1,3-dichloropropene (Trans isomer): Only 4 test concentrations were plotted (not 5 required in both the 1975 guidance cited and OECD 203). Of the four data points one showed 0% mortality, one showed 100% mortality and two data points where partial mortality was observed. No confidence intervals are calculated for the LC_{50} which was a requirement in both the 1975 guidance cited and OECD 203. Despite the deficiencies, greater confidence can be assigned to the results of the trans isomer testing as one partial mortality was observed <35% and one above >65% which was in line with the guidance available at the time of study conduct. Therefore, the results for (E)-1,3-dichloropropene (Trans isomer) from this study could be considered reliable with restriction.

Two acute toxicity studies, conducted with two different species, are available to assess the acute toxicity of 1,3-dichloropropene (Telone II) to fish. One test was conducted with the freshwater salmonid Rainbow trout (*Oncorhynchus mykiss*) and a second test was conducted with the marine cyprinid Sheepshead minnow (*Cyprinodon variegatus*). These studies were conducted under GLP standards according to internationally accepted guidelines (e.g., OECD and USEPA). Fish in both tests were exposed to 1,3-dichloropropene for 96-hours in flow-through diluter systems. Exposure concentrations were confirmed via analytical evaluation. Both tests met current acceptability criteria for fish acute toxicity tests and were considered acceptable by both the Member State Poland as submitter of the CLH dossier and the RMS member state review (DAR, Spain, Volume 3 - B.9 (AS), July 2018).

Two acute toxicity studies, conducted with two different species are available to assess the acute toxicity of (Z)-1,3-dichloropropene (Cis isomer) to fish. One test was conducted with the freshwater salmonid Rainbow trout (*Salmo gairdneri*) and a second test was conducted with the marine cyprinid Sheepshead minnow (*Cyprinodon variegatus*). The Rainbow trout study was conducted under GLP standards according to EPA and EC guidance at the time of study conduct; the study conduct and reporting was considered to be similar the current OECD 203 guidance with only minor reporting deficiencies noted. The Sheepshead minnow study was not conducted to GLP, did not meet the validity conditions of the EPA guidance available at the time of study conduct and has major deficiencies compared to the current OECD 203 guideline; as such it is not considered reliable.

One acute toxicity study, conducted with (E)-1,3-dichloropropene (Trans isomer) and the species sheepshead minnow, *Cyprinodon variegatus*, had a number of deficiencies in comparison to the current guidance, however the number of datapoints required for statistical analysis was sufficient, therefore results could be considered reliable with restriction.

The data from the reliable studies is summarised in the table below; the lowest endpoint which is subsequently used for classification is from the acute Sheepshead minnow study with (E)-1,3-dichloropropene (Trans isomer).

Table 92: Summary of acute toxicity to fish endpoints

Trophic level	1,3-dichloropropene (Mixed isomer)	(Z)-1,3-dichloropropene (Cis isomer)	(E)-1,3-dichloropropene (Trans isomer)
Fish	Rainbow trout (*Oncorhynchus mykiss)* 96-h LC50: 2.78 mg/L **Reliable** Reference: Anonymous 91 (2001)	Rainbow trout (*Salmo gairdneri*) 96h LC50 = 1.6 mg/L **Reliable** Reference: Anonymous 87, 1989	N/A
	Sheepshead minnow (*Cyprinodon variegatus*) 96-h LC50: 0.87 mg/L **Reliable** Reference: Anonymous 92 (1999)	Sheepshead minnow results **not reliable**	Sheepshead minnow (*Cyprinodon variegatus*) 0.290 ppm (0.29 mg/L) **Reliable with restriction** Reference: Anonymous 88, 1978

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Table 93: Summary of relevant information on acute toxicity to aquatic invertebrates

Method	Species	Test material	Results[1]	Remarks	Reference
Aquatic invertebrates					
OECD 202	*Daphnia magna*	Telone II (1,3-Dichloropropene, 96.6%)	48-hour EC50 (immobility): 1.94 mg as/L (95% C.I. 1.30-2.71), based on mean measured concentrations	48-hour static-renewal exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable,**	Anonymous 95, 2013 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.4.1/2

				validity criteria were met	
OECD 202	*Daphnia magna*	Telone II (1,3-Dichloropropene, 96.4%)	48-hour EC50 (immobility): 1.83mg as/L ((95% C.I. 1.26-2.66), based on mean measured concentrations	48-hour static-renewal exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met**	Anonymous 96, 2013 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.4.1/3
OECD 202; EC Directive 92/69/EEC; US EPA 72-2	*Daphnia magna*	Telone II (1,3-Dichloropropene, 100%)	48-hour EC50 (immobility): 3.58mg as/L (95% C.I. 3.35-3.82), based on mean measured concentrations	48-hour static exposure in sealed vessels with zero headspace; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met**	Anonymous 97, 2001 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.4.1/1
US EPA OPPTS 850.1025	Eastern oyster (*Crassotrea virginica*)	Telone II (1,3-Dichloropropene, 96%)	96-hour EC50 (shell growth inhibition): 0.64 mg as/L (95% C.I. 0.58-0.70), based on mean measured concentrations	96-hour flow-through exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable** Key endpoint for classification	Anonymous 89, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.4.2/1
US EPA OPPTS 850.1035	Saltwater mysid (*Mysidopsis bahia*, currently classified as *Americamysis bahia*)	Telone II (1,3-Dichloropropene, 96%)	96-hour LC50: 0.67 mg as/L, based on mean measured concentrations	96-hour flow-through exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met**	Anonymous 98, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.4.2/2
OECD 202;	*Daphnia magna*	Metabolite	48-hour EC50 (immobility): 2.30 mg 3-	48-hour static exposure;	Anonymous 99, 1999

EC Directive 92/69/EEC; US EPA 72-2		3-chloroprop-2-en-1-ol; Chloroallyl Alcohol (3-CAA)	CAA/L based on mean measured concentrations	Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met**	DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.4.1/4
OECD 202; EC Directive 92/69/EEC; US EPA 72-2	*Daphnia magna*	Metabolite 3-chloroacrylic acid (3-CACA)	48-hour EC50 (immobility): 55 mg 3-CACA/L based on mean measured concentrations	48-hour static renewal exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met**	Anonymous 100, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.4.1/5

[1] Indicate if the results are based on the measured or on the nominal concentration.

Five acute toxicity studies, conducted with three different species, are available to assess the acute toxicity of 1,3-dichloropropene (Telone II) to aquatic invertebrates. Three tests were conducted with the freshwater daphnid crustacean *Daphnia magna*, one test was conducted with the marine mysid crustacean *Americamysis bahia* (saltwater shrimp) and a test was conducted with the marine mollusc *Crassotrea virginica* (Eastern oyster). All studies were conducted under GLP standards according to internationally accepted guidelines (e.g., OECD and USEPA). Daphnids in all three tests were exposed to 1,3-dichloropropene for 48-hours under either static (one test) or static-renewal (daily renewal) conditions (two tests). Both the mysid shrimp and Eastern oyster exposures were conducted for 96-hours in flow-through diluter systems. Exposure concentrations in all five tests were confirmed via analytical evaluation. All tests met current acceptability criteria for the relevant toxicity testing guidelines and were considered acceptable by both the Member State Poland as submitter of the CLH dossier and Spain as the RMS for the EFSA review (Draft Assessment Report, Spain, Volume 3 - B.9 (AS), July 2018).

One acute toxicity study, on (Z)-1,3-dichloropropene (Cis isomer) and the species *Daphnia magna*, was conducted under GLP standards to the available EPA and EC guidance at the time and is considered to meet the validity criteria of the current OECD 202 guideline. The 48 h EC_{50} value for *Daphnia magna* test with (Z)-1,3-dichloropropene (Cis isomer) was calculated to be 1.4 mg/L.

The below summary is for the lowest reliable acute endpoint for 1,3-Dichloropropene.

Anonymous 89 (1999)

Title Telone II: A 96 hour shell deposition test with the Eastern oyster (*Crassotrea virginica*).

Guidelines US EPA Series (1996). Series 850 Ecological effects test guidelines (draft), OPPTS No. 850.1025: Oyster acute toxicity test (shell deposition).

ASTM (1994). Standard Guide for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729 88a. American Society for Testing and Materials.

GLP Yes. Laboratory inspected by United States EPA, 401 M Street SW, Washington DC 20460, USA.

METHODOLOGY

Telone II. Lot No. KA10162771, Identification 4610. Purity: 1,3-D content 96%.

The acute toxicity of Telone II to the Eastern oyster (*Crassotrea virginica*) was determined in a 96-hour shell deposition test.

A primary stock solution was prepared in dimethylformamide (DMF) and additional stocks were prepared by dilution. The five stock solutions or DMF, were injected into diluter mixing chambers and mixed with unfiltered seawater to give five nominal test concentrations, one saltwater and one solvent control. Where present, the concentration of DMF was 0.1 ml/L. The diluter delivered 37 volume additions of test water to each test chamber every 24 hours. Test chambers consisted of 52 L Teflon®-lined stainless steel aquaria filled with 13 L test solution. The covered chambers were placed in a temperature controlled water bath (22 ±1°C). Water for holding and testing was natural seawater diluted to 20% salinity with well water.

Prior to testing recently deposited shell was removed from the oysters selected for testing by grinding the periphery with an electric grinder. Groups of twenty oysters (27-39 mm in length) were exposed in test chambers to five nominal test concentrations (0.52, 0.86, 1.4, 2.4, and 4.0 mg Telone II/L), a seawater and solvent control. Mean measured concentrations were determined from samples taken from each chamber at 0, 48 and 96 hours. Concentrations of *cis*- *trans*-1,3-D were summed and adjusted to correct for the 1,3-D content (96%) of Telone II. Oysters were observed at *ca* 2.5, 24, 48, 72 and 96 hours for mortality or clinical signs of toxicity. The longest finger of new shell growth was measured to the nearest 0.05 mm using callipers.

Dissolved oxygen and pH were measured in test chambers at 0, 48 and 96 hours, temperature at 0 and 96 hours, and salinity at 0 hours. Temperature was also measured continuously in a negative control chamber.

The EC_{50} (the concentration estimated to inhibit shell deposition by 50%) was determined using linear interpolation. Evaluation of shell growth measurements indicated that the data were neither normally distributed nor homogeneous, Wilcoxin's rank sum test was used to identify treatments groups with reduced shell growth, compared to pooled controls.

FINDINGS

During testing dissolved oxygen was in the range 6.5-7.7 mg/L (≥84% saturation), pH was 7.9-8.3 and temperature was 21.2-23.0°C. Salinity at 0 hours was 20%. Mean measured concentrations of Telone II at initiation, 48 hours and termination were 4.1-38%, 41-66% and 42-59% of nominal, respectively. It was suggested that low recoveries in test solutions was due to volatility.

Oysters in all Telone II treatment groups, and negative control were reported to be normal and healthy throughout the testing. Compared to shell growth in the pooled control groups, growth was inhibited in treatment groups exposed to measured concentrations of 0.60 mg Telone II/L, or higher. No significant reduction in shell growth was reported at measured concentrations of 0.37 mg Telone II/L, or lower, although there was some slight indication of a concentration-related effect at this concentration.

Table 94: Shell deposition and shell growth inhibition

Nominal concentration (mg Telone II/L)	Mean measured concentration (mg Telone II/L[1])	Shell deposition mm (mean ± SD)[3]	Shell growth inhibition%
Seawater control	<LOQ	3.74 ± 1.54	-
Solvent control	<LOQ	3.79± 1.31	-
Pooled controls	<LOQ	3.77± 1.41	-
0.52	0.28[2]	3.77± 1.11	0.0
0.86	0.37	3.71± 0.98	1.6

1.4	0.60	2.18*± 0.61	42
2.4	0.92	0.90*± 0.45	76
4.0	1.9	0.023*± 0.10	99

<LOQ - < limit of quantitation of 0.252 mg Telone II/L
* significant difference from pooled controls using Wilcoxin's rank sum test ($p \leq 0.05$)
[1]mean of 0h, 48h, 96h measurements of *cis*- and *trans*-1,3-D summed and corrected for 1,3-D purity of Telone II (96%)
[2]the 0h value was <LOQ and extrapolated
3n=20 for treatment groups, n=40 for pooled controls

The 96h EC_{50} was 0.67 mg Telone II/L (95% confidence limits 0.60-0.73 mg/L). The corresponding values for 1,3-D were 0.64 mg/L (0.58-0.70 mg/L). The 96h NOEC was 0.37 mg Telone II/L (0.36 mg 1,3-D/L).

Table 95: Results

	mg/L, measured (95% confidence limits)	
	Telone II	**1,3-D**
96h EC_{50}	0.67 (0.60-0.73)	0.64 (0.58-0.70)
96h NOEC	0.37	0.36

CONCLUSIONS

The 96h EC_{50} value for Eastern oyster (*Crassotrea virginica*) exposed to Telone II was 0.67 mg/L (95% confidence limits 0.60-0.73 mg/L), as measured, based on shell growth inhibition. When expressed as 1,3-D the 96h EC_{50} was 0.64 mg/L (0.58-0.70 mg/L). The 96h NOEC, based on measured values, was 0.37 mg Telone II/L (0.36 mg 1,3-D/L).

As a result, the data from the endpoints derived from these tests are sufficient to consider in the classification determination. The 48-hour EC50 values derived, based on immobility, from the three daphnid tests with 1,3-dichloropropene ranged from 1.83 to 3.58 mg /L. The 96-hour LC50 value for the saltwater mysid shrimp test with 1,3-dichloropropene was 0.67 mg as/L and the 96-hour EC50 value, based on inhibition of shell growth, was 0.64 mg as/L.

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

Table 96: Summary of relevant information on acute toxicity to algae or other aquatic plants

Method	Species	Test material	Results[1]	Remarks	Reference
Algae or other aquatic plants					
OECD 201; OCSPP 850.4500	*Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata,* also formerly known as *Selenastrum capricornutum*)	1,3-Dichloropropene, 96.6% (XRM-5048)	96-hour ErC50: 6.40 mg as/L (95% C.I. 6.28-6.53) based on geometric mean measured concentrations EbC50: 3.48 mg as/L (95% C.I. 3.42-3.54)	96-hour static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable,**	Anonymous 101, 2013 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.1/2

			based on geometric mean measured concentrations EyC50: 3.35 mg as/L (95% C.I. 3.29-3.41) based on geometric mean measured concentrations	**validity criteria were met**	
OECD 201; OCSPP 850.4500	*Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata,* also formerly known as *Selenastrum capricornutum*)	1,3-Dichloropropene, 96.4% (GF-3035)	96-hour ErC50: 5.45 mg as/L (95% C.I. 5.32-5.58) based on geometric mean measured concentrations EbC50: 3.36 mg as/L (95% C.I. 3.27-3.46) based on geometric mean measured concentrations EyC50: 3.41 mg as/L (95% C.I. 3.27-3.56) based on geometric mean measured concentrations	96-hour static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met**	Anonymous 102, 2013 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.1/3
US EPA 123-2, equivalent to EC Directive 92/69/EEC. Guideline C3	*Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata,* also formerly known as *Pseudokirchneriella subcapitata*)	Telone II (1,3-Dichloropropene, 96%)	96-hour EC50: 13.6 mg as/L (95% C.I. <0.029-30.6), based on initial measured values	96-hour static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Not acceptable when reevaluated against updated guideline criteria**	Anonymous 103, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.1/1
US EPA 123-2	*Navicula pelliculosa* (freshwater diatom)	Telone II (1,3-Dichloropropene, 96%)	120-hour EC50 EFSA recalculated proposed: 2.53 mg as/L, based on initial measured 120-hour EC50 Study author: 0.28 mg as/L (95% C.I. <0.018->21.9),	120-hour static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion:	Anonymous 104, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/1

			based on time weighted average measured concentrations	**Not acceptable when reevaluated against updated guideline criteria**	
US EPA 123-2	*Anabaena flos-aquae* (freshwater cyanobacteria or blue-green alga)	Telone II (1,3-Dichloropropene, 96%)	120-hour EC50 EFSA recalculated proposed: 62.58 mg as/L, based on initial measured 120-hour EC50 Study author: 15.5 mg as/L (95% C.I. 6.92-24.0), based on time weighted average measured concentrations	120-hour static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Not acceptable when reevaluated against updated guideline criteria**	Anonymous 105, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/2
US EPA 123-2	*Skeletonema costatum* (saltwater diatom)	Telone II (1,3-Dichloropropene, 96%)	120-hour EbC50 EFSA recalculated proposed: 13.4 mg as/L, based on initial measured concentrations 120-hour EC50 Study author: 12.7 mg as/L (95% C.I. 0.39-25.0), based on time weighted average measured concentrations	120-hour static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Not acceptable when reevaluated against updated guideline criteria**	Anonymous 106, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/3
OECD 221; USEPA OCSPP 850.4400	*Lemna gibba* (freshwater duckweed)	GF-3035 (1,3-Dichloropropene, purity 97.4%)	7-day ErC50: >1.0 mg as/L, based on geometric mean concentrations	7-day daily static-renewal exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met, the endpoint for conducting the risk assessment is pending on the final value submitted by the applicant based on a realistic measured concentration approach.**	Anonymous 107, 2017 DAR, Spain, Volume 3 - B.9 (AS), July 2018 B.9.2.7/02

US EPA 123-2	*Lemna gibba* (freshwater duckweed)	Telone II (1,3-Dichloropropene, 96%)	14-day EC50 Study author: 3.46 mg as/L (95% C.I. 1.79-6.68)	14-day static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Not acceptable when reevaluated against updated guideline criteria**	Anonymous 108, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.7/1
US EPA 123-2	green alga, *Selenastrum capricornutum*	Metabolite 3-chloroacrylic acid (3-CACA)	96-hour ErC50: 1.746 mg 3-CACA/L as mean measured values 96 hour EbC50: 0.663 mg 3-CACA/L as mean measured values	4 day exposure Acceptability conclusion: **The study should be considered as supplementary data.**	Anonymous 109, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.1/4
US EPA 123-2	green alga, *Selenastrum capricornutum*	Metabolite 3-chloroprop-2-en-1-ol (3-CAA)	96-hour ErC50 > 98.0 mg 3-CAA/L as initial measured values 96 hour EbC50: 55.5 mg 3-CAA/L as initial measured values	4 day exposure Acceptability conclusion: **The study should be considered as supplementary data.**	Anonymous 110, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.1/5
US EPA 123-2	saltwater diatom, *Skeletonema costatum*	Metabolite 3 chloroprop 2 en 1 ol (3-CAA)	120 hour ErC50: 0.637 mg 3-CAA/L as initial measured values 120 hour EbC50: 0.492 mg 3-CAA/L as initial measured values	5 day exposure Acceptability conclusion: **Not acceptable when reevaluated against updated guideline criteria.**	Anonymous 111, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/4
US EPA 123-2	saltwater diatom, *Skeletonema costatum*	Metabolite 3-chloroacrylic acid (3-CACA)	120 hour ErC50: 72.3 mg 3-CACA/L as measured values 120 hour EbC50: 56.2 mg 3-CACA/L	5 day exposure Acceptability conclusion: **The study should be considered as supplementary data**	Anonymous 112, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/8

			as measured values		
US EPA 123-2	bluegreen alga, *Anabaena flos-aquae*	Metabolite 3-chloroprop-2-en-1-ol (3-CAA)	120 hour EC50 > 47.5 mg 3-CAA/L as initial measured values	5 day static exposure Acceptability conclusion: **The study should be considered as supplementary data**	Anonymous 113, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/5
US EPA 123-2	freshwater diatom, *Navicula pelliculosa*	Metabolite 3-chloroacrylic acid (3-CACA)	120 hour ErC50: 10.6 mg 3-CACA/L as measured values 120 hour EbC50: 7.09 mg 3-CACA/L as measured values	5 day static exposure Acceptability conclusion: **The study should be considered as supplementary data**	Anonymous 114, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/6
US EPA 123-2	Bluegreen alga, *Anabaena flos-aquae*	Metabolite 3-chloroacrylic acid (3-CACA)	120 hour ErC50 > 12.4 mg 3-CACA/L as measured values 120 hour EbC50: 3.63 mg 3-CACA/L as measured values	5 day static exposure Acceptability conclusion: **The study should be considered as supplementary data**	Anonymous 115, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/7
US EPA 123-2	*Lemna gibba* (freshwater duckweed)	Metabolite 3-chloroprop-2-en-1-ol (3-CAA)	14 day ErC50 > 2.767 mg 3-CAA/L as measured values 14 day EbC50: 0.484 mg 3-CAA/L as measured values	14 day static exposure Acceptability conclusion: **Not accepted**	Anonymous 116, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.7/2
US EPA 123-2	*Lemna gibba* (freshwater duckweed)	Metabolite 3-chloroacrylic acid (3-CACA)	14 day ErC50 > 3.45 mg 3-CACA/L	14 day static exposure Acceptability conclusion: **Acceptable but**	Anonymous 117, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018

			14 day EbC50: 0.28 mg 3-CACA/L 14 day EyC50: 0.454 mg 3-CACA/L	**pending the validity criteria fulfilment.**	CA 8.2.7/3

[1] Indicate if the results are based on the measured or on the nominal concentration.

Eight aquatic plant growth inhibition studies are available to assess the acute toxicity of 1,3-dichloropropene (Telone II). Note that toxicity tests with algae and other aquatic plants don't determine classic acute and chronic endpoints. Testing for the standard species used in toxicity testing covers many generations of the life cycles of these species, and the primary endpoints are based on inhibition of growth or generation time. For risk assessment and classification purposes, different endpoints determined within the same test are used for fulfilling regulatory requirement for acute and chronic endpoints. Acute endpoints are typically filled using an EC50 value, which is generally determined based on inhibition of growth rate but can also be based on inhibition of yield or biomass. Chronic endpoints are typically fulfilled using the NOEC or EC10 or EC20 values based on growth inhibition determined in these same studies.

The eight tests available include testing on five different species of aquatic plants, including four algal species and one aquatic macrophyte species. Six of the tests were conducted with various freshwater and marine algal species while two tests were conducted with duckweed, a freshwater aquatic macrophyte.

Of the six algal toxicity tests, three tests were conducted with the Sphaeropleale green alga, *Raphidocelis subcapitata*, a freshwater green algal species that is the predominate algal test species for toxicity evaluations of potential aquatic toxins. The scientific name has recently been changed from *Pseudokirchneriella subcapitata* (this name is still the scientific name listed in most current testing guidelines) and prior to that it was known as *Selenastrum capricornutum*. Two additional tests were conducted with freshwater species from different phyla, the diatom *Navicula pelliculosa* (Ochrophyta) and the cyanobacteria *Anabaena flos-aquae* (Cyanobacteria), both standard toxicity test organisms for crop protection chemicals. The final algal test was conducted with the marine diatom *Skeletonema costatum*, another standard test organism for crop protection chemicals. All studies were conducted under GLP standards according to internationally accepted guidelines (e.g., OECD and USEPA). The exposures ranged from 96 hours to 120 hours. The 120-hour exposure period was recommended in older USEPA guidelines. Exposure concentrations in all five tests were confirmed via analytical evaluation. Two tests with the green alga *Raphidocelis subcapitata* (Anonymous 101, 2013 and Anonymous 102, 2013) met current acceptability criteria for the relevant toxicity testing guidelines and were considered acceptable by both the Member State Poland as submitter of the CLH dossier and by the EFSA RMS review (Draft Assessment Report, Spain, Volume 3 - B.9 (AS), July 2018). When evaluated against current guidelines, the EFSA RMS reviewer determined that the one green alga *Raphidocelis subcapitata* (Kirk et al. 1999), two diatom and one cyanobacteria study endpoints were not acceptable but did recalculate endpoints ((Draft Assessment Report, Spain, Volume 3 - B.9 (AS), July 2018)). *Navicula pelliculosa* was the most sensitive algal species, with a 120-hour EC50 of 2.53 mg as/L. The endpoint range of the green algae test with 1,3-dichloropropene was 5.45 to 13.6 mg/L and these were considered sufficient for us in classification considerations. The range of EC50s for all algal species ranged from 2.53 to 62.58 mg as/L.

One acute toxicity study, conducted (Z)-1,3-dichloropropene (Cis isomer) and the species *Selenastrum capricornutum*, was conducted under GLP standards to the available EPA and EC guidance at the time and is considered to be highly similar to the current OECD 201 guideline. The 24-72h ErC50 of 3.1 mg/L is therefore the selected acute endpoint for Selenastrum capricornutum, with a NOEC of 0.82 mg/L exposed to for Cis-1,3-Dichloropropene.Two tests were conducted with the aquatic macrophyte *Lemna gibba* (a species of duckweed). The first test, reported in 1999, was a 14-day static exposure test and was considered not acceptable by both the Member State Poland as submitter of the CLH dossier and the RMS reviewer when compared to current guideline acceptability criteria. The EC50 was recalculated by the RMS reviewer and determined to be 41.5 mg as/L. The most recent test (2017) was a 7-day static-renewal exposure with renewals on a daily basis. This test was considered acceptable by both the Member State Poland as submitter of the CLH

dossier and the EFSA RMS reviewer ((Draft Assessment Report, Spain, Volume 3 - B.9 (AS), July 2018)) but the endpoint for conducting the risk assessment is pending on the final value submitted by the applicant based on a realistic measured concentration approach.

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

No relevant tests for classification purposes are reported for 1,3-dichloropropene.

11.6 Long-term aquatic hazard

The summary of the aquatic toxicity studies evaluated during Annex I inclusion of 1,3-dichloropropene and submitted for the purposes of EU renewal is reported below. Only information considered adequate, reliable and relevant for the classification proposal has been included.

The available chronic toxicity data for relevant metabolites of 1,3-dichloropropene (3 chloroprop-2-en-1 ol (3-CAA) and 3-chloroacrylic acid (3-CACA)) revealed toxicity values similar to the parent substance. Therefore, the studies with these metabolites are not described here in detail.

11.6.1 Chronic toxicity to fish

Table 97: Summary of relevant information on chronic toxicity to fish

Method	Species	Test material	Results[1]	Remarks	Reference
Fish					
Fish early life stage toxicity test (OECD 210; USEPA E-540/86-138)	Fathead Minnow, *Pimephales promelas* Rafinesque	Telone II (XRM-5048; 1,3-Dichloropropene, 96.8%)	33 day NOEC: 0.015 mg as/L (based on growth in terms of dry weight) 33 day EC10: 0.020 mg/L (based on growth in terms of dry weight)	33-day flow-through exposure; Analytical confirmation of test substance Acceptability conclusion: Acceptable, validity criteria were met Key endpoint for classification	Anonymous 90, 2015 DAR, Spain, July 2018 CA 8.2.4.1/3
Fish early life stage toxicity test (OECD 210; USEPA E 540/86 138; ASTM Standard E 1241 92)	Fathead Minnow, *Pimephales promelas* Rafinesque	Telone II (1,3-Dichloropropene, 96%)	33 day NOEC: 0.032 mg as/L (based on mortality) EFSA evaluation (agreed on by RMS) NOEC Study author: 0.117 mg as/L	33-day flow-through exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Accepted**	Anonymous 118, 2000 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.2.1/1
Fish early life stage toxicity test (OECD 210)	Fathead Minnow, *Pimephales promelas*	Metabolite 3-chloroacrylic acid (3-CACA)	33-d NOEC = 2.22 mg 3-CACA/L mean measured concentration	33-day flow-through exposure; Acceptability conclusion: Accepted	Anonymous 119, 2007 DAR, Spain, Volume 3 - B.9 (AS), July 2018

[1] Indicate if the results are based on the measured or on the nominal concentration

The below summary is for the lowest chronic endpoint, this is subsequently used for classification assessment.

Anonymous 90 (2015)

Title	XRM-5048: An Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales promelas*).
Guidelines	OECD Guideline 210, fish early life stage toxicity test. EPA FIFRA. Hazard Evaluation Division, Standard Evaluation Procedure; fish early life stage. E 540/86 138.
GLP	Yes.

MATERIALS AND METHODS

Test Item(s)

Purity:	96.8% (49.9% cis 1,3-D, 46.9% trans 1,3-D)

Test System

Organism (*Species*):	Fathead minnow (*Pimephales promelas*)
Study type:	Early-Life Stage
Duration of study:	33 days
Test conditions:	Flow through
Parameters measured:	Time to hatch, hatching success, survival and growth (total length, wet weight and dry weight)
Observation intervals:	Twice during the first day of exposure and once daily thereafter until test end
Stage of embryonic development at test initiation:	Less than 24 hours old between stage 8 (gastrula) and stage 9 (neurula)
Test concentrations:	Nominal: 6.3, 13, 25, 50, 100 µg/L Mean measured: 3.8, 8.7, 15, 34, 49 µg/L
Analytical confirmation of test concentrations:	On days: -7, 0, 7, 14, 21, 28, 33
Reference substance:	Not applicable
Brood stock holding conditions:	Not applicable
No. of holding days before dosing:	Not applicable
Number of eggs per dose group:	80 (4 replicates each containing 20 embryos)
Number of eggs per control group:	80 (4 replicates each containing 20 embryos)
Feeding regime:	Newly hatched larvae were fed live brine shrimp nauplii three times per day during the first seven days of post-hatch, thereafter, larvae were fed live brine shrimp nauplii three times per day on weekdays and at least two times per day on weekends.

Environmental conditions:	Loading rate: 0.018 g fish/L of test solution that passed through the test chamber during a 24-hour period, 0.32 g fish/L (the total wet weight of fish per liter of water in the tank) Temperature: 25 ± 1°C Photoperiod: 16 hours of light and 8 hours of darkness Dissolved oxygen concentration: ≥100% of saturation (≥8.2 mg/L) pH: 7.7 to 8.1 Total hardness: 132 to 136 mg/L as $CaCO_3$ in the dilution water control, 134 to 140 mg/L as $CaCO_3$ in the highest test concentration solution

Methodology

Fathead minnow embryos were exposed to a geometric series of five test concentrations, a negative (dilution water) control and a solvent control (0.1 mL/L HPLC-grade dimethylformamide) under flow-through conditions. The exposure period included a 5 day embryo hatching period, and a 28-day post-hatch juvenile growth period. Nominal test concentrations were 6.3, 13, 25, 50 and 100 µg/L. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, at weekly intervals during the test and at test termination.

Delivery of the test solutions to the test chambers was initiated two days prior to test initiation in order to achieve equilibrium of the test substance. Four replicate test chambers were maintained in each treatment and control group, with one incubation cup in each test chamber. Each incubation cup contained 20 embryos, resulting in a total of 80 embryos per treatment. At test initiation, embryos <24 hours old were impartially distributed to incubation cups and exposed to test solution in the test chambers. After a 5 day embryo hatching period, the larvae were released into the test chambers, where exposure continued during a 28-day post-hatch juvenile growth period. Observations of the effects of XRM-5048 on time to hatch, hatching success, survival, length, wet weight and dry weight in comparison to the pooled control group were used to calculate the no-observed-effect-concentration (NOEC), the lowest-observed-effect-concentration (LOEC), and the effective concentrations for an x percent effect (ECx), typically EC10 and EC20, were calculated, when possible.

Two sets of water samples were collected from alternating replicate test chambers of each treatment and control group 7 days prior to test initiation to confirm the operation of the diluter system and during the test on Days 0, 7, 14, 21, 28 and 33 (test termination) to determine concentrations of the test substance in the test chambers. All samples were collected at mid-depth in the test chambers, placed in glass vials with septum cap and one set was processed immediately for analysis. The samples were diluted in freshwater, as necessary, and submitted for analysis by gas chromatography with flame ionization detection (GC/FID) using an Archon/Tekmar purge and trap sampling system.

RESULTS AND DISCUSSION

Analytical verification of XRM-5048 concentrations in the test solution collected during the test on Days 0, 7, 14, 21, 28 and 33 had percent recoveries ranged from 45.1 to 77.8% of nominal. Mean measured test concentrations were 3.8, 8.7, 15, 34 and 49 µg/L, which represented 60, 67, 62, 68 and 49% of nominal concentrations, respectively. The majority of embryos hatched on Days 4 and 5 of the test and all viable embryos hatched on day 6 of the test. Hatching success in the negative control and solvent control were both 99%. Hatching success in the 3.8, 8.7, 15, 34 and 49 µg/L treatment groups was 100, 100, 99, 100 and 100%, respectively. There were no statistically significant differences in hatching success in any of the XRM-5048 treatment groups when compared to the pooled controls. The NOEC for hatching success was 49 µg/L and the LOEC was greater than 49 µg/L. The EC10 and EC20 values and the corresponding 95% confidence

intervals for hatching success were not reported since the ECx values were not bracketed by the test concentrations used for the calculation and/or the 95% confidence intervals were overly wide.

Percent survival of larvae in the negative control and solvent control was 94 and 90%, respectively. Larval survival in the 3.8, 8.7, 15, 34 and 49 µg/L treatment groups was 91, 95, 94, 96 and 84%, respectively. There were no statistically significant differences in larval survival in any of the XRM-5048 treatment groups when compared to the pooled controls The NOEC for larval survival was 49 µg/L and the LOEC was greater than 49 µg/L. The EC10 was 48 µg/L, with the corresponding 95% confidence interval of 41 to 49 µg/L. The EC20 values and its corresponding 95% confidence interval were not reported since the EC20 value was not bracketed by the test concentrations used for the calculation and thus could only be extrapolated and/or the 95% confidence intervals were overly wide.

The mean total length in the negative control and solvent control were both 25.0 mm. The mean wet weight in the negative control and solvent control was 111.4 and 116.3 mg, respectively, while the mean dry weight was 22.6 and 23.2 mg, respectively. Mean total length in the 3.8, 8.7, 15, 34 and 49 µg/L treatment groups was 24.7, 25.4, 25.1, 25.1 and 24.0 mm, respectively. Mean wet weight in the 3.8, 8.7, 15, 34 and 49 µg/L treatment groups was 103.1, 112.9, 108.9, 104.8 and 93.6 mg, respectively. Mean dry weight in the in the 3.8, 8.7, 15, 34 and 49 µg/L treatment groups was 20.1, 22.6, 21.0, 20.1 and 18.6 mg, respectively. There was not a significant concentration related trend in total length (Jonckheere-Terpstra Trend test, $p > 0.05$). However, a significant concentration related trend was found in the 49 and 34 µg/L treatment concentrations in wet weight and dry weight, respectively. The additional Dunnett's one-tailed test indicated statistically significant reductions in total length among fish in the 49 µg/L treatment group in comparison to the pooled controls ($p \leq 0.05$). A significant difference in mean wet weight were found at the 3.8, 34 and 49 µg/L treatment groups and in mean dry weight at the 3.8, 15, 34 and 49 µg/L treatment groups when compared to the pooled controls (Dunnett's one-tailed test, $p \leq 0.05$). However, the mean wet weight and dry weight in the 3.8 µg/L treatment group did not follow a concentration-response pattern so is not considered biologically meaningful. A difference in mean dry weight at the 15 µg/L treatment group from the pooled controls was detected by Dunnett's test. However, the effect was not detected in wet weight or length using Dunnet's analysis. In addition, the significant differences noted were not detected in the Jonckheere-Terstra trend test. Consequently, growth measured as dry weight was the most sensitive endpoint of the test. The NOEC and LOEC for growth (measured as dry weight) were 15 and 34 µg/L, respectively.

Table 98: Effects of test item on hatchability, survival and growth of fathead minnow

Treatment (µg/L)	No. of eggs at study initiation	% Egg hatchability	No. of surviving fry	% Fry survival	Mean length of surviving fish (mm)	Mean wet weight of surviving fish (mg)	Mean dry weight of surviving fish (mg)
Negative control	80	99	74	94	25.0	111.4	22.6
Solvent control	80	99	71	90	25.0	116.3	23.2
3.8	80	100	73	91	24.7	103.1$^{\Delta}$	20.1$^{\Delta}$
8.7	80	100	76	95	25.4	112.9	22.6
15	80	99	74	94	25.1	108.9	21.0$^{\Delta}$
34	80	100	77	96	25.1	104.8$^{\Delta}$	20.1*,$^{\Delta}$
49	80	100	37	84	24.0	93.6*,$^{\Delta}$	18.6*,$^{\Delta}$
	Hatchability		Survival		Growth		
EC_{10} (µg/L)	NA		48		20		
EC_{20} (µg/L)	NA		NA		NA		
NOEC (µg/L)	>49		49		15		
LOEC (µg/L)	>49		>49		34		

Treatment (µg/L)	No. of eggs at study initiation	% Egg hatchability	No. of surviving fry	% Fry survival	Mean length of surviving fish (mm)	Mean wet weight of surviving fish (mg)	Mean dry weight of surviving fish (mg)
MATC (µg/L)	>49		>49		23		

* Indicates a significant difference in wet and dry weight (Jonkheere-Terpstra trend test, p≤0.05) from the pooled controls.

Δ Indicates a statistically significant difference in total length, wet and dry weight (Dunnett's one-tail-test, p≤0.05) from the pooled controls. However, the differences in wet and dry weight in the 3.8 µg/L treatment group did not follow a dose-response pattern. In addition, the difference in mean dry weight at the 15 µg/L treatment group from the pooled controls was slight (8.3%). Therefore, the significant difference in mean wet weight at 3.8 µg/L treatment concentration and in mean dry weight detected at the 3.8 and 15 µg/L treatment concentrations were not considered biologically meaningful.

CONCLUSION

Fathead minnows (*Pimephales promelas*) were exposed to XRM-5048 at mean measured concentrations of 3.8, 8.7, 15, 34 and 49 µg/L under flow-through conditions for 33 days (a 5-day hatching period plus a 28-day post-hatch growth period). The study met all conditions for the validity of the test stated in OECD guideline 210. There were no statistically significant, treatment-related effects on hatching success or survival at concentrations <49 µg/L. Growth (measured as mean dry weight) was the most sensitive biological endpoint measured in this study. Fathead minnows exposed to XRM-5048 at a concentration of 34 µg/L had statistically significant reductions in growth in comparison to the pooled controls. Consequently, the NOEC, LOEC and MATC for growth was 15, 34 and 23 µg/L, respectively.

Summary of available studies

Two chronic toxicity studies are available to assess the chronic toxicity of 1,3-dichloropropene (Telone II) to fish. Both tests were conducted with the freshwater cyprinid Fathead minnow (*Pimephales promulas*). These studies were conducted under GLP standards according to internationally accepted guidelines (OECD 210 and USEPA). Technically, the OECD 210 Guideline (FELS) is not a 'chronic' test, but a sub-chronic test on sensitive life stages. It is widely accepted as a predictor of chronic toxicity and is used as such for purposes of classification in the harmonised system. Fish eggs and larvae in both tests were exposed to 1,3-dichloropropene for 33-days in flow-through diluter systems. Exposure concentrations were confirmed via analytical evaluation. Both tests met current acceptability criteria for fish early life stage toxicity tests and were considered acceptable by both the Member State Poland as submitter of the CLH dossier and the RMS review (Draft Assessment Report, Spain, Volume 3 - B.9 (AS), July 2018). As a result, the data from the endpoints derived from these tests are sufficient to consider in the classification determination. The 33-day NOEC values derived from these two tests ranged from 0.015 to 0.032 mg as/L. In the 2015 study appropriate statistical analysis determined the EC_{10} value of 0.020 mg as/L.

11.6.2 Chronic toxicity to aquatic invertebrates

Table 99: Summary of relevant information on chronic toxicity to aquatic invertebrates

Method	Species	Test material	Results[1]	Remarks	Reference
Aquatic invertebrates					
US EPA 72-4, equivalent to OECD Guideline No. 202, Part II	*Daphnia magna*	Telone II (1.3-Dichloropropene, 96 %)	NOEC: 0.0701 mg as/L, based on mean measured concentrations (standard)	21-day flow-through exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion:	Anonymous 120, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.5.1/1

				Acceptable, validity criteria were met	
OECD 219	*Chironomus riparius*	Telone II (1.3-Dichloropropene, 97.4%)	28-day NOEC: 0.369 mg as/L, based on initial measured concentrations (standard for this study type)	28-day static exposure; Test vessels were sediment-water systems and test material was introduced to the test vessels via overlying water; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met**	Anonymous 121, 2017 DAR, Spain, Volume 3 - B.9 (AS), July 2018 B.9.2.5.3/01
OECD Guideline 211; USEPA OPPTS 850.1300.	*Daphnia magna*	Metabolite 3-chloroacrylic acid (3-CACA)	21-day NOEC: 2.53 mg as/L, based on mean measured concentrations	21-day static-renewal exposure Acceptability conclusion: **Acceptable, validity criteria were met**	Anonymous 122, 2007 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.5.1/2

[1] Indicate if the results are based on the measured or on the nominal concentration

One chronic toxicity study is available to assess the chronic toxicity of 1,3-dichloropropene (Telone II) to aquatic invertebrates. This study was conducted under GLP standards according to an internationally accepted guideline (OECD 202, Part II). Daphnid neonates were exposed to 1,3-dichloropropene for 21-days in flow-through diluter system. Exposure concentrations were confirmed via analytical evaluation. The test met current acceptability criteria for daphnid chronic reproduction tests and was considered acceptable by both the Member State Poland as submitter of the CLH dossier and Spain as the RMS for the EFSA review. As a result, the data from the endpoints derived from these tests are sufficient to consider in the classification determination. The 21-day NOEC value derived from this test was 0.0701 mg as/L.

11.6.3 Chronic toxicity to algae or other aquatic plants

Table 100: Summary of relevant information on chronic toxicity to algae or other aquatic plants

Method	Species	Test material	Results[1]	Remarks	Reference
Algae or other aquatic plants					
OECD 201; OCSPP 850.4500	*Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata,* also formerly known as *Selenastrum*	1,3-Dichloropropene, 96.6% (XRM-5048)	96-hour NOEC: 0.889 mg as/L	4-day static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable,**	Anonymous 123, 2013 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.1/2

	capricornutum)			**validity criteria were met**	
OECD 201; USEPA OCSPP 850.4500	*Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata,* also formerly known as *Selenastrum capricornutum*)	1,3-Dichloropropene, 96.4% (GF-3035)	96-hour NOEC: 0.759 mg as/L	4-day static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met**	Anonymous 124, 2013 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.1/3
US EPA 123-2, equivalent to EC Directive 92/69/EEC. Guideline C3	*Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata,* also formerly known as *Pseudokirchneriella subcapitata*)	Telone II (1,3-Dichloropropene, 96%)	NOEC: 3.44 mg as/L, based on time weighted average measured concentrations	96-hour static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Not acceptable when reevaluated against updated guideline criteria**	Anonymous 125, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.1/1
US EPA 123-2	*Navicula pelliculosa* (freshwater diatom)	Telone II (1,3-Dichloropropene, 96%)	120-hr NOEC: <0.018 mg as/L based on time weighted average measured concentrations	120-hour static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Not acceptable when reevaluated against updated guideline criteria**	Anonymous 126, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/1
US EPA 123-2	*Anabaena flos-aquae* (freshwater cyanobacteria or blue-green alga)	Telone II (1,3-Dichloropropene, 96%)	120-hr NOEC: 2.75 mg as/L, based on time weighted average measured concentrations	120-hour static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Not acceptable when reevaluated against updated guideline criteria**	Anonymous 127, 1999, DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/2
US EPA 123-2	*Skeletonema costatum* (saltwater diatom)	Telone II (1,3-Dichloropropene, 96%)	120-hr NOEC: 2.47 mg as/L), based on time weighted average measured concentrations	120-hour static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Not**	Anonymous 128, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/3

				acceptable when reevaluated against updated guideline criteria	
OECD 221; USEPA OCSPP 850.4400	*Lemna gibba* (freshwater duckweed)	GF-3035 (1,3-Dichloropropene, purity 97.4%)	7-day NOEC: 0.071 mg as/L, based on geometric mean concentrations EC10: 0.18 mg as/L, from Biomass Growth Rate, based on geometric mean concentrations	7-day daily static-renewal exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met, the endpoint for conducting the risk assessment is pending on the final value submitted by the applicant based on a realistic measured concentration approach.**	Anonymous 129, 2017 DAR, Spain, Volume 3 - B.9 (AS), July 2018 B.9.2.7/02
US EPA 123-2	*Lemna gibba* (freshwater duckweed)	Telone II (1,3-Dichloropropene, 96%)	14-day NOEC Study author: 0.845 mg as/L, based on time weighted average measured concentrations	14-day static exposure; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Not acceptable when reevaluated against updated guideline criteria**	Anonymous 130, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.7/1
US EPA 123-2	green alga, *Selenastrum capricornutum*	Metabolite 3-chloroacrylic acid (3-CACA)	96-hour NOEC: 0.183 mg/L as mean measured values	4 day exposure Acceptability conclusion: **The study should be considered as supplementary data.**	Anonymous 131, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.1/4
US EPA 123-2	green alga, *Selenastrum capricornutum*	Metabolite 3-chloroprop-2-en-1-ol (3-CAA)	96-hour NOEC: 0.0127 mg 3-CAA/L as mean measured values	4 day exposure Acceptability conclusion: **The study should be considered as supplementary data.**	Anonymous 132, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.1/5
US EPA 123-2	saltwater diatom, *Skeletonema costatum*	Metabolite 3 chloroprop 2 en 1 ol (3-CAA)	120 hour NOEC: 0.0707 mg 3-CAA/L	5 day exposure Acceptability conclusion:	Anonymous 133, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018

				Not acceptable when reevaluated against updated guideline criteria.	CA 8.2.6.2/4
US EPA 123-2	saltwater diatom, *Skeletonema costatum*	Metabolite 3-chloroacrylic acid (3-CACA)	120 hour NOEC: 23.9 mg 3-CACA/L	5 day exposure Acceptability conclusion: **The study should be considered as supplementary data**	Anonymous 134, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/8
US EPA 123-2	bluegreen alga, *Anabaena flos-aquae*	Metabolite 3-chloroprop-2-en-1-ol (3-CAA)	120 hour NOEC: 47.5 mg 3-CAA/L as initial measured values	5 day static exposure Acceptability conclusion: **The study should be considered as supplementary data**	Anonymous 135, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/5
US EPA 123-2	freshwater diatom, *Navicula pelliculosa*	Metabolite 3-chloroacrylic acid (3-CACA)	120 hour NOEC: 2.59 mg 3-CACA/L as measured values	5 day static exposure Acceptability conclusion: **The study should be considered as supplementary data**	Anonymous 136, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/6
US EPA 123-2	Bluegreen alga, *Anabaena flos-aquae*	Metabolite 3-chloroacrylic acid (3-CACA)	120 hour NOEC: 3.40 mg 3-CACA/L as measured values	5 day static exposure Acceptability conclusion: **The study should be considered as supplementary data**	Anonymous 137, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.6.2/7
US EPA 123-2	*Lemna gibba* (freshwater duckweed)	Metabolite 3-chloroprop-2-en-1-ol (3-CAA)	14 day NOEC< 7.66 mg 3-CACA/L	14 day static exposure Acceptability conclusion: **Not accepted.**	Anonymous 138, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.7/2
US EPA 123-2	*Lemna gibba (freshwater duckweed*	Metabolite 3-chloroacrylic acid (3-CACA)	14 day NOEC < 0.016 mg 3-CACA/L	14 day static exposure Acceptability conclusion: **Acceptable but pending the validity criteria fulfilment.**	Anonymous 139, 1999 DAR, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.7/3

[1] Indicate if the results are based on the measured or on the nominal concentration

See section 11.5.3 for a summary of the algae and aquatic plant studies. Acute endpoints from these studies are presented in section 11.5.3. Chronic endpoint (i.e., NOEC) values for these studies are summarized here. NOEC values for the green alga *Raphidocelis subcapitata* ranged from 0.759 to 3.44 mg as/L and were considered sufficient to consider for classification determination. NOEC values for all algae ranged from <0.018 mg as/L (*Navicula pelliculosa*) to 3.44 mg as/L (*Raphidocelis subcapitata*). NOEC values for the aquatic macrophyte Lemna gibba ranged from 0.071 to 0.845 mg as/L and were considered sufficient to consider for classification determination.

11.6.4 Chronic toxicity to other aquatic organisms

Table 101: Summary of relevant information on chronic toxicity to other aquatic organisms

Method	Species	Test material	Results[1]	Remarks	Reference
Aquatic invertebrates					
OECD 219	*Chironomus riparius*	Telone II (1.3-Dichloropropene,97 .4%)	28-day NOEC: 0.369 mg as/L, based on initial measured concentrations (standard for this study type) The EC10, based on the development rate of midges exposed to water spiked with GF-3035 was 1.20 mg a.i./L with 95% confidence limits of 0.441 to 3.29 mg a.i./L	28-day static exposure; Test vessels were sediment-water systems and test material was introduced to the test vessels via overlying water; Analytical confirmation of test substance concentrations; Acceptability conclusion: **Acceptable, validity criteria were met**	Anonymous 140, 2017 DAR, Spain, Volume 3 - B.9 (AS), July 2018 B.9.2.5.3/01

[1] Indicate if the results are based on the measured or on the nominal concentration

A sub-chronic toxicity study that assessed the effect of 1,3-dichloropropene (Telone II) on the emergence of *Chironomus riparius* in a water/sediment exposure system. This study was conducted under GLP standards according to internationally accepted guidelines (e.g., OECD and USEPA). First instar *C. riparius* were exposed to overlying water spiked with test material in a water-sediment system and they were monitored for 28 days for emergence. Exposure concentrations were confirmed via analytical evaluation. The test met current acceptability criteria for the sediment-water chironomid toxicity test and was considered acceptable by the RMS member state review. The 28-day NOEC value derived from this test was 0.369 mg as/L. While this study can inform the assessment, the daphnia chronic test is more applicable for classification purposes.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Several aquatic toxicity tests evaluating the acute toxicity of 1,3-dichloropropene to various species from three trophic levels determined the most acutely sensitive species tested was the marine mollusc *Crassotrea virginica* (Eastern oyster) with a 96-hour EC50 of 0.64 mg as/L based on the shell growth inhibition endpoint. This endpoint value was in a similar range to the endpoints derived from testing with two other species, a 96-hour LC50 of 0.67 mg as/L for the marine mysid crustacean *Americamysis bahia*

(saltwater shrimp) and a 96-hour LC50 0.87 for the marine fish Sheepshead minnow (*Cyprinodon variegatus*). The lowest result from the proposed group was for (E)-1,3-dichloropropene (Trans isomer) with the species the Sheepshead minnow, *Cyprinodon Variegatus*, resulting in an LC_{50} of 0.29mg/L. The CLP Regulation defines short-term (acute) aquatic criteria for classification. Endpoint values ≤ 1 mg as/L for either fish, crustacea or algae/aquatic plants would be categorized as Category Acute 1, which is consistent with the acute data generated for 1,3-dichloropropene.

PROPOSED CLASSIFICATION:

Based on data presented, 1,3-D should be classified in accordance with the CLP Regulation as:

Aquatic acute toxicity Category 1, H 400, Very toxic to aquatic life. The M factor is 1 as described in section 11.8.

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

Several aquatic toxicity tests evaluating the chronic toxicity of 1,3-dichloropropene to various species from three trophic levels determined the most acutely sensitive species tested was the Fathead minnow (*Pimephales promelas*), with a 33-day NOEC of 0.015 mg as/L and an EC_{10} of 0.020 mg as/L.

The ready biodegradability of 1,3-dichloropropene was investigated using OECD 301D Closed-Bottle Test. The active ingredient did not degrade beyond 5% during the 28 day incubation period and would be classified as not readily biodegradable. However, there were several studies conducted that evaluated the potential for 1,3-dichloropropene to degrade by abiotic and biotic processes. DT50 values derived from hydrolysis studies in sterile buffered solution ranged from 2.69 to 4.75 days. Hydrolysis testing in non-sterile water from a natural source produced a DT50 of 11.1 days. DT50 values from further testing in representative US water/sediment systems ranged from 4.5 to 4.9 days. Testing in four representative European soils indicated that 1,3-dichloropropene was rapidly dissipated from test soils with DT50 values ranging from 8.8 to 15 days, indicating rapid degradation and dissipation under aerobic conditions, with microbial degradation, hydrolysis and volatilisation as the major routes of dissipation. Based on this information, 1,3-dichloropropene is expected to rapidly degrade in aquatic systems and terrestrial soils. The conditions of CLP Section 4.1.2.9.3 to fulfil the criteria of rapid degradability are not met. The full mineralisation condition is not achieved in the aforementioned environmental fate studies therefore, 1,3-dichloropropene should be assessed as not rapidly degradable.

The log Kow for 1,3-dichloropropene ranged from 1.82 to 2.1 at 20°C, based on testing with both the cis and trans isomers. Due to a log Kow ≤4, as well as rapid removal from the environment, bioconcentration and subsequent bioaccumulation through the food chain is not expected.

The CLP Regulation defines long-term (chronic) aquatic classification criteria. For substances that are not rapidly degradable and have chronic endpoint values ≤ 0.1 mg as/L for either fish, crustacea or algae/aquatic plants. A classification of Category Chronic 1 would be assigned based on the EC_{10} value of 0.020 mg as/L which is consistent with the chronic data generated for 1,3-dichloropropene.

PROPOSED CLASSIFICATION:

Based on data presented, 1,3-D should be classified in accordance with the CLP Regulation as:

Aquatic chronic toxicity Category 1, H 410, Very toxic to aquatic life with long lasting effects. The M factor is 1 as described in section 11.8.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Based on the *Cyprinodon Variegatus* LC_{50} equal to 0.29 mg/L *Aquatic acute toxicity Category 1, H 400, Very toxic to aquatic life* with a M factor = 1 is proposed.

Based on the *Pimephales promelas* NOEC equal to 0.015 mg/L *Aquatic chronic toxicity Category 1, H 410, Very toxic to aquatic life with long lasting effects* with a M factor = 1 is proposed.

The CLP Regulation also outlines the assignment of M-factors for consideration of the toxicity contribution of 1,3-dichloropropene if used as a component (at levels outlined in the guidance). As a result, the following M-factors are proposed for 1,3-dichloropropene:

CLP Range Acute toxicity (mg/L) L(E)C50 value	CLP M factor	1,3-DCP Lowest L(E)C50 (mg/L)	Proposed Acute M factor
0.1 < L(E)C50 ≤ 1	1	0.29	**1**
0.01 < L(E)C50 ≤ 0.1	10	not applicable	not applicable
Chronic toxicity (mg/L) **NOEC value**	**CLP** **M factor**	**1,3-DCP** **Lowest NOEC (mg/L)**	**Proposed Chronic** **M factor**
0.01 < NOEC ≤ 0.1	1	0.015	1
0.001 < NOEC ≤ 0.01	10	not applicable	not applicable

Note: The chronic M factor is defined in table 4.1.3 of CLP based on the NOEC which has been quoted in this table. It is noted that EC_{10} values are generally preferred for classification purposes as regression based estimates are less influenced by dose selection. The EC_{10} value from the same study is 0.020 mg as/L which would lead to the same M factor proposal.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Ozone layer

1,3-Dichloropropene is a highly volatile compound (H > 100 Pa m3 mol^{-1}). Its volatility is medium based on a boiling point between 50ºC and 150ºC (103.8-105.2ºC for the cis-isomer and 114.5ºC for the trans-isomer) and a vapour pressure between 0.5-25 kPa (4.850 kPa for the cis-isomer and 2.982 kPa for the trans-isomer at 25ºC). Volatilisation will be the major route of dissipation in the environment.

One study is available to assess the Ozone Depletion Potential (ODP) of 1,3-dichloropropene (Anonymous 141 (1997)). ODP of 1,3 D has been conducted under GLP standards and evaluated using the Dow Chemistry Climate Model (DOWCCM). The study was considered acceptable by both the Member State Poland as submitter of the CLH dossier and Spain as the RMS for the EFSA review.

Assuming an ODP for HCFC-123 of 0.014 DOWCCM computed an ODP for 1,3-D of 0.002. This result reveals the low ODP of 1,3-D.

On the other hand, atmospheric 1,3-D is relatively short lived (half-life of 1 day approx. as a result of indirect photo oxidation reactions (Anonymous 142, (2014)).

Moreover, its atmospheric breakdown products formyl chloride and chloroacetaldehyde (Anonymous 143, (1984)) would also be efficiently removed from the lower troposphere, as they are water soluble or react in solution to form water soluble products (i.e. they will be re-deposited on land or in the oceans). It can be concluded that the breakdown products would be very short lived in the atmosphere.

Therefore, 1,3-D is unlikely to have any detrimental effect on the stratospheric ozone layer.

Ozone Depleting Potential (DPP) of 1,3-D at 0.002 is lower than the lowest ODP (0.005) of substances currently listed in Annex I to Regulation (EC) No 2024/590 (repealing Regulation (EC) No 1005/2009) and is not classified as hazardous to the ozone layer.

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14 ANNEXES

Annex I (Non-confidential Annex): Justification for Applying a Grouping Approach for the Classification of 1,3-dichloropropene (mixture of cis and trans isomers), (Z)-1,3-dichloropropene (cis isomer) and (E)-1,3-dichloropropene (trans isomer) in Accordance with Regulation (EC) No. 1272/2008 (the CLP Regulation)

Annex II (Confidential Annex): Full reference list including study authors; see separate document.

14.1 Annex I: Justification for Applying a Grouping Approach for the Classification of 1,3-dichloropropene (mixture of cis and trans isomers), (Z)-1,3-dichloropropene (cis isomer) and (E)-1,3-dichloropropene (trans isomer) in Accordance with Regulation (EC) No. 1272/2008 (the CLP Regulation)

The following document provides an evaluation of the available information and experimental data for 1,3-dichloropropene (mixture of cis and trans isomers, referred to as "1,3-D (mix of isomers)"), (Z)-1,3-dichloropropene (cis isomer, referred to as "cis 1,3-D") and (E)-1,3-dichloropropene (trans isomer, referred to as "trans 1,3-D") as part of a read-across, category approach for the justification for grouping these substances in one group for the purposes of establishing a harmonized classification according to Regulation (EC) No. 1272/2008 (the CLP Regulation). On the basis of this evaluation, a grouping and read-across approach has been following in the CLH proposal for these substances.

A group or category of substances may be defined for those members that have physicochemical, toxicological and ecotoxicological properties that are likely to be similar or follow a regular pattern as a result of structural similarity. Classification for the group overall is based on read-across from data available for the category members in a conservative manner (i.e. taking the worst case hazard outcome overall). Applying the grouping concept means that information for physicochemical, human health and/or environmental properties may be predicted from information from tests conducted on reference substance(s) within the group through read-across.

The group considered for this CLH proposal covers 1,3-dichloropropene (mixture of isomers), (Z)-1,3-dichloropropene (cis isomer) and (E)-1,3-dichloropropene (trans isomer). 1,3-dichloropropene (mixture of isomers) and (Z)-1,3-dichloropropene (cis isomer) currently have an entry in Annex VI (Number 602-030-00-5) with the following classifications: Flam. Liq. 3 (H226); Acute Tox. 3 (H301); Acute Tox. 3 (H311); Skin Irrit. 2 (H315); Eye Irrit. 2 (H319); Skin Sens. 1 (H317); Acute Tox. 4 (H332); Asp. Tox. 1 (H304); STOT SE 3 (H335); Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410). The applicant proposes to expand this group to include (E)-1,3-dichloropropene (trans isomer). All the potential group members have been included in the group and the available data have been considered in this proposal.

The proposed read-across approach is considered according to the ECHA Guidance Document for categories, Guidance on information requirements and chemical safety assessment, Chapter R.6: QSARs and grouping of chemicals (ECHA, 2008). The Read-Across Assessment Framework (RAAF) (ECHA, 2017) has also been used as a reference. For further discussion of the category approach see the Category definition section below.

Background

1,3-dichloropropene is proposed for approval as an active substance in the meaning of Regulation EC 1107/2009 therefore is subject to harmonised classification and labelling according to Article 36 CLP Regulation.

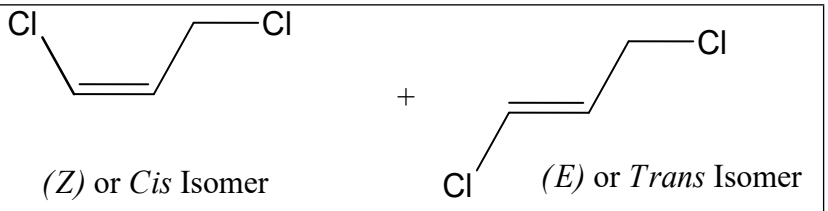
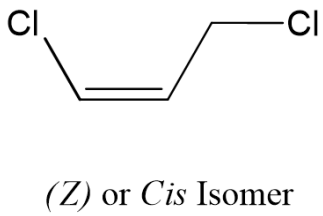
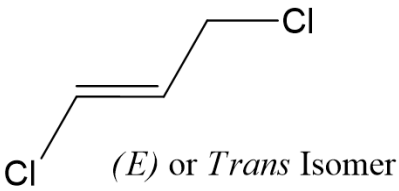
Category definition

The structures covered by the group proposal are stereoisomers. One of the category members 1,3-dichloropropene (EC 208-826-5) has been extensively tested as a plant protection active substance and is a mixture of the two isomers also included in the category.

The table below identifies the substances which form the proposed category.

Table A1: Substances included in the proposed category

Name	EC number (CAS)	Structure(s)

1,3-dichloropropene	208-826-5 (542-75-6)	
(Z)-1,3-dichloropropene (cis isomer)	233-195-8 (10061-01-5)	
(E)-1,3-dichloropropene (trans isomer)	431-460-4 (10061-02-6)	

Category hypothesis

Properties of category members may be predicted from reference substances within the category based on structural similarity.

Formation of identical and noncommon compounds is discussed in the relevant sections along with subsequent degradation where applicable.

The reliability and adequacy of the available studies have been assessed. Where testing is available on more than one category member within the physical chemistry, human health, or environmental sections these have been assessed in a data matrix to compare the properties. The produced data matrices are used to verify the validity and robustness of the category hypothesis. Where conclusions differ read-across from a category member is conducted in a conservative manner (worst case).

Physical-chemical properties

A read across matrix has been prepared for physical-chemical properties of 1,3-D (mix of isomers), cis 1,3-D and trans 1,3-D providing a comparison of data and conclusions (study references are provided in the CLH dossier).

Table A2: Data matrix for physical properties of 1,3-dichloropropene (mixture of cis and trans isomers), (Z)-1,3-dichloropropene (cis isomer) and (E)-1,3-dichloropropene (trans isomer)

Property	1,3-dichloropropene (Mixed isomer)	(Z)-1,3-dichloropropene (Cis isomer)	(E)-1,3-dichloropropene (Trans isomer)
Physical state at 20°C and 101,3 kPa	Technical: clear colourless liquid with odour of chlorinated solvents. Anonymous 3, 1998	cis-isomer: slightly yellow clear liquid Anonymous 1, 1990	trans-isomer: clear colourless liquid at 20C with odour of chlorinated solvent. Anonymous 2, 1998

Property	1,3-dichloropropene (Mixed isomer)	(Z)-1,3-dichloropropene (Cis isomer)	(E)-1,3-dichloropropene (Trans isomer)
Melting/freezing point		cis-isomer: freezing point – 85 ºC (188 K) Anonymous 1, 1990	trans-isomer: < –25 ºC (lowest temperature achieved in the test). Anonymous 2, 1998
Boiling point		cis-isomer: 103.8 – 105.2 ºC Anonymous 1, 1990	trans-isomer: 114.5 ºC. Anonymous 2, 1998
Relative density		cis-isomer: Relative density (D234) = 1.221 Anonymous 1, 1990	trans-isomer: Relative density (D234) = 1.23 Anonymous 2, 1998
Vapour pressure		cis-isomer: 3760 Pa at 20ºC 4850 Pa at 25ºC Anonymous 1, 1990	trans-isomer: 2982 Pa at 25ºC Anonymous 2, 1998
Surface tension		cis-isomer: 69.6 ± 0.4 mN/m at 20 °C (90% saturated solution) - not surface active Anonymous 4, 2005	trans-isomer: 61.0 mN/m at 21 °C (1 g/L solution) - not surface active Anonymous 2 1998
Water solubility		cis-isomer (20 ºC): 2.45 g/L Anonymous 1, 1990	trans-isomer (20 ºC): 2.52 g/L Anonymous 2, 1998
Partition coefficient n-octanol/water		Log Kow cis-isomer: 1.82 at 20°C Anonymous 1, 1990	Log Kow trans-isomer: 2.1 at 20°C Anonymous 2, 1998
Flash point	Technical: Flash point 27.0 ºC. Anonymous 3, 1998	cis-isomer: Flash point: 28.5 ºC. Anonymous 1, 1990	
Flammability	Technical: Non-flammable (contact with water) Anonymous 3, 1998	cis-isomer does not evolve highly flammable gases on contact with water. Cis-1,3-dichloropropene did not ignite during dropping or within five minutes of setting in any of the replicates tests. Cis-1,3-dichloropropene is non-pyrophoric. Anonymous 1, 1990,	
Explosive properties	Technical: Technical 1,3-dichloropropene is not explosive. Anonymous 3, 1998	cis-isomer does not have any explosive properties. Anonymous 1, 1990	
Self-ignition temperature	Technical: None below 400˚C Anonymous 3, 1998	cis-isomer auto ignition temperature = 555 ± 5 ºC. Anonymous 1, 1990	
Oxidising properties	Non oxidising Anonymous 5, 2005		
Granulometry	n.a.	n.a.	n.a.

Property	1,3-dichloropropene (Mixed isomer)	(Z)-1,3-dichloropropene (Cis isomer)	(E)-1,3-dichloropropene (Trans isomer)
Stability in organic solvents and identity of relevant degradation products	Solvent g/L Methanol > 250 g/L Acetone > 250 g/L Xylene > 250 g/L 1,2-dichloroethane > 250 g/L Ethyl acetate > 250 g/L n-heptane > 250 g/L n-octanol > 250 g/L Anonymous 3, 1998		
Dissociation constant	Not applicable for a non-ionisable compound.	Not applicable for a non-ionisable compound.	Not applicable for a non-ionisable compound.
Viscosity	*Kinematic Viscosity:* 0.636 mm^2s^{-1} at 20°C 0.544 mm^2s^{-1} at 40°C *Dynamic Viscosity:* Newtonian 0.769 mPa.s at 20°C 0.658 mPa.s at 40°C Anonymous 6, 1997		

Where data has been generated on one or more of the proposed category members these are comparable. There are no differences between the category members which would result in different physical hazards being identified under CLP.

Mammalian toxicology

The following section provides an evaluation of the available mammalian toxicity data for 1,3-dichloropropene (mixture of cis and trans isomers, referred to as "1,3-D (mix of isomers)"), (Z)-1,3-dichloropropene (cis isomer, referred to as "cis 1,3-D") and (E)-1,3-dichloropropene (trans isomer, referred to as "trans 1,3-D") as part of a read-across, category approach for the justification for grouping these substances in one group for the purposes of establishing a harmonized classification according to Regulation (EC) No. 1272/2008 (the CLP Regulation).

The corresponding toxicological data matrix is shown in Table A3.

Toxicokinetics

The toxicokinetic profile of 1,3-D (mix of isomers) is described in detail the 2018 DAR Volume II Chapter 3, B6 (AS)): a brief overview only has therefore been provided for the purposes of supporting the read-across justification.

Toxicokinetic studies evaluating the absorption, tissue distribution, metabolism and elimination of 1,3-D have demonstrated that the compound is quickly and extensively absorbed both through the respiratory tract and the gastrointestinal tract, is widely distributed throughout the body, extensively metabolized and rapidly excreted. The toxicokinetic profile of 1,3-D is comparable in humans and in animals.

1,3-D undergoes nearly complete metabolism in mammalian species in the liver via a major pathway involving direct glutathione (GSH) conjugation catalysed by glutathione S-transferase (GST) resulting in the formation of a mercapturic acid metabolite (N-acetyl-(S-3-chloroprop-2-enyl)cysteine (3CNAC) and several secondary metabolites that are rapidly excreted via the urine. Thus, the major metabolic pathway for 1,3-D leads to its rapid detoxification and excretion. A minor pathway for the metabolism of 1,3-D involves hydrolysis with dechlorination resulting in the formation of intermediates that are substrates for alcohol dehydrogenase. A further third "trace" pathway has been identified as oxidation mediated by cytochrome P450 resulting in the formation of reactive 1,3-D epoxide species. This trace pathway is only considered to occur at very high doses (i.e.: approximately the LD_{50}) when the major metabolic detoxification pathways are likely to be overwhelmed. Elimination of 1,3-D is very rapid, irrespective of the route of absorption. On the basis that 1,3-D is rapidly absorbed and extensively metabolized and excreted, the compound is unlikely to accumulate in the body.

In two respective oral toxicokinetic studies conducted using radio-labelled cis 1,3-D and trans 1,3-D, similar profiles of metabolism and elimination were observed for the two isomers. In the study using cis 1,3-D, six Carworth Farm E strain rats were administered cis-1,3-dichloro(2-^{14}) propene (2.51 mg, 7.98 µCi in 0.5 ml of arachis oil) via oral gavage whereas in the study using trans 1,3-D, twelve rats were administered trans-1,3-dichloro[2-^{14}C]propene orally. Excretion via the urine and faeces was observed for 4 days and any remaining radioactivity in the gut, skin and carcass was analysed following the sacrifice of the rats. In both studies respectively, cis 1,3-D and trans 1,3-D were rapidly metabolised. Following the administration of cis 1,3-D, the primary route of excretion was via the urine (83.1%) with 80.7% of the administered dose eliminated within 24 hrs. Minor amounts were eliminated via the faeces (2.6%) and as carbon dioxide (3.8%). Following the administration of trans 1,3-D, the primary route of elimination was via the urine (58.0% over 4 days), whereas 23.5% was eliminated as carbon dioxide and 2.2% via the faeces. In both studies respectively, only traces of radioactivity remained in rats after 4 days.

In a non-GLP study of the metabolism of cis 1,3-D, two female Wistar rats were each given a single oral dose of cis-1,3-dichloro(2-^{14})propene (ca. 5mg, approximately 20 mg/kg bodyweight) in corn oil (0.7ml) and the metabolites in the urine and the faeces were analysed. The major urinary metabolite of cis 1,3-D was identified as N-acetyl-S-[(Z)-3-chloroprop-2-enyl]cysteine (the mercapturic acid of (Z or cis)-DCP). Cis 1,3-D was also shown to react with glutathione in the presence of rat liver cytosol to produce S(Z)-3-chloroprop-2-enyl]glutathione.

In conclusion, based on the available experimental data, 1,3-D (mix of isomers), cis 1,3-D and trans 1,3-D have comparable toxicokinetic profiles hence a category approach for the classification of endpoints in respect of human health is considered to be justified.

Acute toxicity

Acute oral toxicity

In acute oral toxicity studies conducted according to OECD TG 401 (or comparable methods i.e.: EEC Method B1), the LD_{50} values in rats were determined to be 150, 85-121 and 94 mg/kg bw for 1,3-D (mix of isomers), cis 1,3-D and trans 1,3-D respectively (Anonymous 8 (1986), Anonymous 9 (2006), Anonymous 17 (1988), Anonymous 23 (1989) and Anonymous 18 (1988)).

According to the criteria of Regulation (EC) No. 1272/2008 (the CLP Regulation): substances for which the acute oral LD_{50} is > 50 mg/kg and ≤ 300 mg/kg bw require classification in Acute Toxicity Category 3 (H301) *"Toxic if swallowed."*

In conclusion, the evaluation of available experimental data on 1,3-D (mix of isomers), cis 1,3-D and trans 1,3-D indicates that these substances have a comparable profile with respect to the potential for acute oral toxicity. These substances respectively meet the CLP criteria for classification in Acute Toxicity Category 3 (H301) "*Toxic if swallowed."* Based on a category approach, the grouping of these substances for classification in respect of this endpoint is therefore considered to be appropriate.

Acute dermal

In acute dermal toxicity studies conducted according to OECD TG 402 (or comparable methods i.e.: EEC Method B3), the LD_{50} values in rats were determined to be 1200 and 1575 mg/kg for 1,3-D (mix of isomers) and trans 1,3-D respectively (Anonymous 10 (1986), Anonymous 18 (1988)). In two respective rat studies using cis 1,3-D conducted in accordance with OECD TG 402, LD_{50} values of 794 and 1090 mg/kg bw were determined (Anonymous 19 (1988), Anonymous 23 (1989)). In an acute dermal toxicity study conducted in rabbits using 1,3-D (mix of isomers), the LD_{50} was determined to be 333 mg/kg bw/day (Anonymous 11 (1987)).

According to the criteria of the CLP Regulation: substances for which the acute dermal LD_{50} is > 1000 mg/kg and ≤ 2000 mg/kg bw require classification in Category 4 for acute dermal toxicity, whereas substances for which the acute dermal LD_{50} is > 200 and ≤ 1000 mg/kg bw require classification in Category 3 for acute dermal toxicity.

In conclusion, the evaluation of the available experimental data on 1,3-D (mix of isomers) and cis 1,3-D respectively indicates that these substances have a comparable profile of acute dermal toxicity and meet the CLP criteria for classification in Acute Toxicity Category 3 (H311) *"Toxic in contact with skin."* While the available data for trans 1,3-D meet the criteria for classification in Acute Toxicity Category 4 (H312) *"Harmful in contact with skin,"* the overall classification of the group of substances in Category 3 in respect of acute dermal toxicity would be considered to be precautionary. Based on a category approach, the grouping of these substances for classification in respect of this endpoint is therefore considered to be appropriate.

Acute inhalation

In two acute inhalation toxicity studies conducted according to OECD TG 403 in which rats received whole body exposures to vapours of 1,3-D (mix of isomers), the 4-hour inhalation LC_{50} values ranged from 2.70-3.07 mg/L and from 3.88-4.70 mg//L respectively (Anonymous 12 (1987), Anonymous 13 (1987)).

In an acute inhalation toxicity study conducted according to EPA Guideline 81-3 in which rats received whole body exposures to cis 1,3-D, the 4-hour inhalation LC_{50} values were determined to be 670 ppm (3.04 mg/L) and 744 ppm (3.38 mg/L) in male and female rats respectively (Anonymous 20 (1990)).

In an acute inhalation toxicity study conducted according to OECD TG 403 in which rats received head only exposures to vapours of trans 1,3-D, the 4-hour inhalation LC_{50} value was determined to be 5.098 (4.54-6.092) mg/L (both sexes), (Anonymous 21 (1989)).

According to the criteria of the CLP Regulation: substances for which the acute inhalation 4-hour LC_{50} (vapours) > 2 and ≤ 10 mg/kg bw require classification in Acute Toxicity Category 3 (H331) *"Toxic if inhaled."*

In conclusion, the evaluation of available experimental data on 1,3-D (mix of isomers), cis 1,3-D and trans 1,3-D indicates that these substances have a comparable profile with respect to the potential for acute inhalation toxicity. These substances respectively meet the CLP criteria for classification in Acute Toxicity Category 3

(H331) "*Toxic if inhaled."* Based on a category approach, the grouping of these substances for classification in respect of this endpoint is therefore considered to be appropriate.

Skin irritation

The skin irritation potential of 1,3-D (mix of isomers) was investigated in six New Zealand White rabbits in a study conducted according to EPA Guideline 81-5 (Anonymous 14 (1987)). Animals were treated with an application of 0.5 mL of undiluted substance to the shaved skin of the back under a semi-occlusive dressing for 4 hours. The mean scores for erythema of each tested animal from gradings at 24, 48 and 72 hours after patch removal were 2.0, 2.0, 1.66, 2.66, 2.66 and 2.33. The mean scores for oedema of each tested animal from gradings at 24, 48 and 72 hours were 1.0, 1.66, 1.33, 1.33, 1.66 and 2.33. Inflammation persisted to the end of the observation period of 14 days in 4 animals with erythema score 2, oedema score from 1 to 2 and exfoliation.

The criterion for classification (CLP Regulation) in Category 2 of skin irritation: "mean score of ≥ 2.3 and ≤ 4.0 for erythema/ eschar or for oedema in at least 2 (4) of 3 (6) i.e.:66.7% of tested animals from gradings at 24, 48 and 72 hours after patch removal" has not been met for 1,3-D. However, the criterion: "Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals" has been met. Hence, on the basis of these findings, 1,3-D (mix of isomers) meets the CLP criteria for classification in Skin irritation Category 2 (H315) *"Causes skin irritation."*

The skin irritation potential of cis 1,3-D was investigated in six New Zealand White rabbits in a study conducted according to OECD TG 404 (Anonymous 23 (1989)). Animals were treated with an application of 0.5 mL of undiluted substance to clipped dorsal skin under a semi-occlusive dressing for 4 hours. The mean scores for erythema of each tested animal from gradings at 24, 48 and 72 hours after patch removal were 1.0, 1.0, 1.33, 1.33, 1.67 and 1.33. The mean scores for oedema of each tested animal from gradings at 24, 48 and 72 hours were 1.33, 1.67, 2, 1.67, 1.0 and 1.33. Dermal desquamation of the treated area was observed in all animals at 7 and at 14 days after treatment and persisted in one animal until day 21.

The criterion for classification (CLP Regulation) in Category 2 of skin irritation: "mean score of ≥ 2.3 and ≤ 4.0 for erythema/ eschar or for oedema in at least 2 (4) of 3 (6) i.e.: 66.7% of tested animals from gradings at 24, 48 and 72 hours after patch removal" has not been met for cis 1,3-D. However, the criterion: "Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals" is considered to be met, on the basis that dermal desquamation, an effect relevant to the consideration of persistent inflammation for classification purposes was observed in all animals at 7 and at 14 days after treatment. Hence, on the basis of these findings, cis 1,3-D meets the CLP criteria for classification in Skin Irritation Category 2 (H315) *"Causes skin irritation."*

The skin irritation potential of trans 1,3-D was investigated in six New Zealand White rabbits in a study conducted according to EEC Method B4 (Anonymous 18 (1988)). Animals were treated with an application of 0.5 mL of undiluted substance to clipped skin under a semi-occlusive dressing for 4 hours. The mean scores for erythema of each tested animal from gradings at 24, 48 and 72 hours after patch removal were 1.67, 1.67, 1.67, 2.0, 2.0 and 1.0. The mean scores for oedema of each tested animal from gradings at 24, 48 and 72 hours were 0.67, 0.67, 0.67, 1.0, 1.0 and 0.33. Dermal desquamation of the treated area was observed in two animals with mean scores for erythema of 1.67 and 2.0 respectively, 14 days after treatment.

The criterion for classification (CLP Regulation) in Category 2 for skin irritation: "mean score of ≥ 2.3 and ≤ 4.0 for erythema/ eschar or for oedema in at least 2 (4) of 3 (6) i.e.: 66.7% of tested animals from gradings at 24, 48 and 72 hours after patch removal" has not been met for trans 1,3-D. However, the criterion: "Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals" is considered to be met, on the basis that dermal desquamation, an effect relevant to the consideration of persistent inflammation for classification purposes was observed in two animals with means scores of 1.67 and 2.0 for erythema, 14 days after treatment. Hence, on the basis of these findings, trans 1,3-D meets the CLP criteria for classification in Skin Irritation Category 2 (H315) *"Causes skin irritation."*

In conclusion, the evaluation on available experimental data, 1,3-D (mix of isomers), cis 1,3-D and trans 1,3-D have a comparable profile with respect to the potential for skin irritation. These substances respectively meet the CLP criteria for classification in Skin Irritation Category 2 (H315) *"Causes skin irritation."* Based on a category approach, the grouping of these substances for classification in respect of this endpoint is therefore considered to be appropriate.

Eye irritation

The eye irritation potential of 1,3-D (mix of isomers) was investigated in six New Zealand White rabbits in a study conducted according to EPA Guideline 81-4 (Anonymous 15 (1987)). Aliquots of 0.1 mL of undiluted test material were instilled into the conjunctival sac of the right eye of each animal. The left eye remained untreated and served as control. The mean scores following grading at 24, 48 and 72 hours after instillation of the test material were: 0.0, 0.0, 1.0, 0.0, 0.0 and 0.0 for corneal opacity; 1.0, 0.0, 1.0, 0.33, 1.0 and 0.0 for iritis; 2.66, 0.66, 3.0, 2.0, 3.0 and 1.66 for conjunctival redness and 2.66, 0.0, 2.66, 1.0, 2.0 and 1.0 conjunctival oedema (chemosis). All signs of eye irritation gradually subsided and were absent 14 days post-treatment.

The criterion for classification (CLP Regulation) in Category 2 for eye irritation: "conjunctival redness ≥ 2.0" in 2/3 (i.e.: 66.7%) animals from gradings at 24, 48 and 72 hours after treatment which fully reversed within 21 days of treatment" was met. Hence, on the basis of this study,1,3-D (mix of isomers) meets the CLP criteria for classification in Eye Irritation Category 2 (H319) *"Causes serious eye irritation."*

The eye irritation potential of cis 1,3-D was investigated in three New Zealand White rabbits in a GLP Isolated Eye Test following the methods described by Price and Andrew (1985) (Anonymous 23 (1989)). Aliquots of 0.1 mL of undiluted test material were applied *ex vivo* to six excised eyes from the rabbits that were clamped in the test chamber for 10 seconds and subsequently irrigated with warm saline. Corneal thickness was measured at hourly intervals after dosing for a total of 5 hours, using a fluorescein dye.

Application of 0.1 mL of undiluted cis 1,3-D to each of six isolated rabbit eye preparations resulted in an increase of corneal thickness by more that 20% (group mean value) within 3 hours of treatment. Corneal uptake of fluorescein was apparent in all preparations following application of the dye to the corneal surface 4 hours after treatment. On the basis of these results *in vitro*, the application of cis 1,3-D *in vivo* is predicted to result in significant ocular damage persisting for 21 days after treatment.

The eye irritation potential of trans 1,3-D was investigated in six New Zealand White rabbits in a study conducted according to EEC Method B5 (Anonymous 18 (1988)). Aliquots of 0.1 mL of undiluted test material were instilled into the conjunctival sac of one eye of each animal. The mean scores following grading at 24, 48 and 72 hours after instillation of the test material were: 1.0, 0.67, 0.67, 0, 0.67 and 1.0 for corneal opacity; 0, 0, 0.33, 0, 0.33 and 0 for iritis; 3.0, 1.67, 1.67, 1.67, 2.0 and 2.67 for conjunctival redness and 1.67, 1.0, 1.0, 1.0, 1.33 and 1.33 for conjunctival oedema (chemosis). Resolution of the eye irritant effects was advanced 7 days after treatment and fully resolved within 14 days.

The criteria for classification (CLP Regulation) in Category 2 for the eye irritation effects: corneal opacity, iritis, conjunctival redness and conjunctival oedema (chemosis) were not met based on gradings at 24, 48 and 72 hours after treatment and irritation effects fully reversed within 21 days of treatment. On the basis of this study, trans 1,3-D does not meet the CLP criteria for classification in respect of eye irritation.

According to the Guidance on the Application of the CLP Criteria (Version 5.0, July 2017), the Isolated Rabbit Eye (IRE) *ex vivo* test can be regarded as a validated test method without an OECD Test Guideline, and positive findings from the test can be taken into consideration as part of a "Top-Down" precautionary approach for the classification of substances in Eye Irritation Category 1 (H319) *"Serious eye damage,"* for example, when no other data are available and for the avoidance of further animal testing, if deemed unnecessary. Since *in vivo*, guideline studies are available for 1,3-D a weight of evidence approach is considered to be appropriate. Overall, 1,3-D (mix of isomers) and cis 1,3-D have demonstratable eye irritation potential whereas trans 1,3-D causes mild irritation effects that do not meet the criteria for classification, hence classification of the group overall as Eye Irritation Category 2 (H319) *"Causes serious eye irritation,"* is considered to be appropriate.

Skin sensitization

The skin sensitization potential of 1,3-D (mix of isomers) was investigated in ten guinea pigs in a Buehler test conducted according to OECD TG 406 in which the animals were induced with the substance at 0.1 % v/v once weekly for 3 weeks and challenged at the same concentration two weeks after the last induction (Anonymous 16 (1987)). Slight to moderate erythema was observed in 9/10 guinea pigs (90%) challenged with 0.1% v/v 1,3-D.

The CLP criteria for classification in Skin Sensitisation Category 1A are met since > 15% of animals tested in the Buehler assay responded at a topical induction dose ≤ 0.2%. Hence on the basis of these findings,1,3-D

(mix of isomers) meets the CLP criteria for classification in Skin Sensitisation Category 1A (H317) *"May cause an allergic reaction."*

The skin sensitization potential of cis 1,3-D was investigated in twelve guinea pigs in a modified Buehler test conducted according to OECD TG 406 in which the animals received 9 induction applications during a 2-week period (Anonymous 22 (1988)). The first 3 inductions used 2% cis 1,3-D, whereas the remaining inductions used the substance at 1%. Two weeks after the last inductions, animals were challenged with 0.5% cis 1,3-D. On challenge, 4/12 (33.3%) animals responded with slight to moderate erythema indicating that cis 1,3-D has skin sensitization potential.

The CLP criteria for classification in Skin Sensitisation Category 1B are met since >15% of animals tested in the Buehler assay responded at a topical induction dose ≥ 0.2% and ≤ 20 %. Hence, on the basis of these findings, cis 1,3-D meets the CLP criteria for classification in Skin Sensitisation Category 1B (H317) *"May cause an allergic reaction."*

In the further study, the skin sensitization potential of cis 1,3-D was investigated in a guinea pig maximization test (GPMT; according to Magnusson and Kligman) in accordance with OECD TG 406 (Anonymous 23 (1989)). In the study 20 animals received an induction dose at 0.1% m/v intradermally, a topical induction dose at 5% m/v and a topical challenge at 2.5% m/v.

Positive responses were observed in 20/20 (100%) of the animals 24 hours after topical challenge and in 18/20 (90%) of the animals after challenge. The CLP criteria for classification in Skin Sensitisation Category 1A are met since ≥ 30% of animals tested in the GPMT responded at an intradermal induction dose ≤ 0.1%. Hence, on the basis of these findings, cis 1,3-D meets the CLP criteria for classification in Skin Sensitisation Category 1A (H317) *"May cause an allergic reaction."*

The skin sensitization potential of trans 1,3-D was investigated in guinea pigs in a study conducted according to EEC Method B6 (Guinea pigs maximization test (GPMT) according to Magnusson and Klingman) in which 20 animals received an induction dose at 0.05% m/v intradermally, a topical induction dose at 10% m/v and a topical challenge at 5% m/v (Anonymous 18 (1988)).

Positive responses were observed in 16/20 (80%) of the animals 24 hours after topical challenge and in 15/20 (75%) of the animals 48 hours after challenge. The CLP criteria for classification in Skin Sensitisation Category 1A are met since ≥ 30% of animals tested in the GPMT responded at an intradermal induction dose ≤ 0.1%. Hence, on the basis of these findings, trans 1,3-D meets the CLP criteria for classification in Skin Sensitisation Category 1A (H317) *"May cause an allergic reaction."*

In conclusion, the evaluation of the available experimental data on 1,3-D (mix of isomers), cis 1,3-D and trans 1,3-D indicates that these substances have a comparable profile with respect to the potential for skin sensitisation. These substances respectively meet the CLP criteria for classification in Skin Sensitisation Category 1A (H317) *"May cause an allergic reaction."* Based on a category approach, the grouping of these substances for classification in respect of this endpoint is therefore considered to be appropriate.

Short-term repeated dose toxicity

The short-term (<28 day) and sub-chronic (90 day) repeated dose toxicity of 1,3-D (mix of isomers) has been investigated in studies conducted in rats, mice and dogs (the studies are summarised in the 2018 DAR Volume II Chapter 3, B6 (AS)).

In 14-day palatability and dietary probe studies conducted in rats, mice and dogs, effects observed were primarily reduced bodyweights and feed consumption. The stomach was the primary target organ in rats. In a 4-week vapour inhalation probe study in SD CD rats in which the animals were exposed to 1,3-D at 90, 150 and 200 ppm, decreased body weights and body weight gains were observed.

In a 13-week dietary toxicity and 4-week recovery study conducted in Fischer F344 rats according to OECD Test Guideline 408 in which the animals received 1,3-D at 0, 5, 15, 50 and 100 mg/kg bw/day, the No-

observed-effect level (NOEL) was determined to be 5 mg/kg bw/day for both sexes based on hyperkeratosis of the stomach and basal cell hyperplasia of the non-glandular mucosa of the stomach.

In a 13-week dietary and 4-week recovery study conducted in B6C3F1 mice according to OECD Test Guideline 408, the LOAEL was determined to be 15 mg/kg bw/day, based on the statistical bodyweight decrease at this dose.

In a one-year dietary toxicity study conducted in Beagle dogs receiving 1,3-D at 0, 0.5, 205 or 15 mg/kg bw/day, the NOAEL was determined to be 2.5 mg/kg bw/day based on decreased body weight, increased liver weight, hypochromic and microcytic anaemia, and increased hematopoiesis in the bone marrow and spleen.

In study-chronic inhalation toxicity studies using 1,3-D, male and female Fischer 344 albino rats and B6C3F1 mice (10 animals/sex/ exposure concentration) were exposed to 1,3-D via the whole body (for 6 hr/day, 5 day/week) at 0, 10, 30, 90 or 150 ppm for a total of 13 weeks. The NOEL was 10 ppm for male rats based on hyperplasia of the respiratory epithelium at 30 ppm, and was 30 ppm for female rats, based on lower levels of serum protein and weight loss observed at 90 ppm. The NOAEL for male mice was 30 ppm based on depressed body weights and dose-related effect on the nasal mucosa and 10 ppm for female mice based on aggregates of mononuclear (predominately lymphoid) cells in submucosa of urinary bladder.

In a 14-day GLP-compliant inhalation study, rats (15/sex/group) were exposed via the whole body to vapours of cis 1,3-D at 0, 10, 60 and 150 ppm for 6 hours/day, 7 days/week for 9 exposures (Anonymous 48 (1990)). The NOAEL was determined to be 60 ppm (male/female) based on changes in body weight, non-protein sulfhydryl levels in the liver, kidneys and lungs and histopathological changes of moderate severity in the respiratory and olfactory mucosa in the nasal cavity in male and female rats exposed at the top dose level of 150 ppm.

In a 90-day sub-chronic inhalation toxicity study according to OECD TG 413, Fischer 344 rats (10/sex/group) were exposed to vapours of cis 1,3-D at 0, 10, 30 and 90 ppm for 6 hours/day, 5 days/week for 13 weeks (Anonymous 49 (1991)). The NOAEL was determined to be 30 ppm (male and female) based on decreased body weight of males and hyperplasia of the nasal and respiratory epithelia of males and females exposed to 90 ppm

In a GLP-compliant inhalation toxicity range-finding study conducted according to OECD TG 412, Wistar rats (5/sex/group) were exposed via nose-only inhalation to vapours of trans 1,3-D at 0, 30, 100 or 300 ppm for 6 hrs/day, 5 days/week for 14 days (Anonymous 50 (1999)). Based on the findings of the study it was concluded that exposures to vapours of trans 1,3-D for 14 days at 30, 100 or 300 ppm was associated with a concentration-related decrease in body weight gain in all male exposure groups and in females of the mid and high concentration groups and, besides some other changes in relative organ weights, in a concentration-related increase in relative lung weight of males of the mid and high concentration group and of females of the high concentration group.

In the subsequent 90-day sub-chronic inhalation toxicity study conducted according to OECD TG 413, Wistar rats (10/sex/group) were exposed via nose-only inhalation to vapours of trans 1,3-D at 0, 10, 30 or 90 ppm (target concentrations: 8.9, 26.9 and 80.7 ppm) for 6 hours/day, 5 days/week for 13 weeks (Anonymous 51 (1999)). The NOAEL was determined to be 8.9 ppm based on decreased body weight gain in animals in the mid and high dose groups. Increased relative liver and kidney weights were observed in animals treated at the high dose level.

Short-term or sub-chronic, repeated dose inhalation studies using cis 1,3-D and trans 1,3-D respectively report changes in body weight gain and liver and kidney affects as systemic effects and respiratory/nasal effects at higher doses.

Repeated dose toxicity studies using 1,3-D (mix of isomers), cis 1,3-D and trans 1,3-D indicate that the toxicity profile of these substances is comparable.

Genotoxicity

Genotoxicity in vitro

Bacterial cells – gene mutation assays

The dataset of bacterial cell gene mutation assays for 1,3-D (mix of isomers) comprises eight studies of mixed quality and reliability: four studies are considered to be unacceptable, three studies provide supplemental information and one OECD TG 472 study is considered to be acceptable (Anonymous 41 (1978), Anonymous 28 (1978), Anonymous 32 (1996), Anonymous 34 (1997), Anonymous 35 (1999), Anonymous 33 (2009), Anonymous 31 (2004), Anonymous 39 (1989)). The weight of evidence indicates that 1,3-D is capable of inducing gene mutations in bacteria with and without a metabolic activation system (i.e.: liver S9 mix).

Four studies have investigated the potential for cis 1,3-D to induce gene mutations in bacterial cells systems. One study is considered to be unacceptable, two provide supplemental information and one study was conducted in according with OECD 471 (Anonymous 28 (1978), Anonymous 42 (1978), Anonymous 43 (1978), Anonymous 44 (1990)). The weight of evidence indicates that cis 1,3-D is capable of inducing gene mutations in bacteria with and without a metabolic activation system (i.e.: liver S9 mix) and provides a comparable profile of mutagenic potential towards bacterial cells as observed for 1,3-D (mix of isomers).

Observations that the mutagenic effects of 1,3-D in bacteria can be eliminated by co-incubation with glutathione, provides useful evidence regarding the role of glutathione in protecting cells from 1,3-D genotoxicity.

No studies are available that have investigated the mutagenic potential of trans 1,3-D in bacterial cell systems.

Mammalian cell – gene mutation assays

In an *in vitro* mammalian cell gene mutation assay conducted according to OECD TG 476, 1,3-D (mix of isomers) did not induce mutations in the HPRT gene of Chinese hamster ovary cells (Anonymous 36 (1986)).

While no studies are available that have evaluated the potential of cis 1,3-D or trans 1,3-D to induce gene mutations in mammalian cells, the absence of positive findings in the study conducted using 1,3-D, a mixture containing 48.9 % cis 1,3-D and 43.2% trans 1,3-D indicates that the respective isomers are not expected to have potential to cause gene mutations in mammalian cells *in vitro.*

Mammalian cell – chromosome aberration assays

In an *in vitro* chromosome aberration study conducted according to OECD TG 473, 1,3-D (mix of isomers) was clastogenic (i.e.: induced structural aberrations) in Chinese hamster lung cells in the presence of and in the absence of a metabolic activation system (S9 mix) and aneugenic (i.e.: induced numeric aberrations) in the absence of a metabolic activation system, after the 48 hr treatment (Anonymous 38 (1988)). Since chromosome aberrations were observed at concentrations that approached or exceeded acceptable levels of cytotoxicity, the results were considered to be of limited reliability.

In an *in vitro* chromosome aberration study conducted according to OECD TG 473 (EPA Guideline 84-2), cis 1,3-D gave positive results in Chinese hamster ovary (CHO-K) cells in the presence of a metabolic activation system (S9 mix) but negative results in the absence of a metabolic activation system (Anonymous 45 (1991)). The addition of exogenous glutathione reduced the clastogenic activity of cis 1,3-D and the associated chromosomal structural damage, suggesting that a clastogenic metabolite generated in the presence of the S9 mix may be detoxified by glutathione at physiologically relevant concentrations.

In an *in vitro* chromosome aberration study conducted according to EPA Guideline 84-2, trans 1,3-D was clastogenic in Chinese hamster ovary (CHO-K1) cells in the presence of a metabolic activation system (rat liver S9 mix) but negative results were obtained in the absence of a metabolic activation system (Anonymous 46 (1989)).

The available experimental data indicate that 1,3-D (mix of isomers), cis 1,3-D and trans 1,3-D have a comparable profile in terms of potential cytogenetic toxicity in mammalian cells *in vitro*, characterized primarily by the induction of chromosomal aberrations in the presence of metabolic activation which may be prevented by the presence of the detoxifying substance, glutathione.

Mammalian cell – DNA damage/DNA binding

In a study of limited reliability, 1,3-D (mix of isomers) gave positive results in an *in vitro* sister-chromatic exchange (SCE) test using human lymphocytes conducted according to OECD TG 479 (Kevekordes *et al.* (1996)).

1,3-D (mix of isomers) did not induce DNA repair in primary rat hepatocytes treated *in vitro* in a study conducted according to OECD TG 482 (Anonymous 37 (1985)).

Negative results were obtained for 1,3-D (mix of isomers) in an *in vitro* DNA binding (adduct) assay using calf thymus cells conducted according to OECD TG 482 (Anonymous 40 (1997)).

No studies are available that have investigated the potential for cis 1,3-D or trans 1,3-D to induce DNA damage or bind to DNA to form adducts.

Genotoxicity *in vivo* (somatic cells)

Mammalian gene mutation assays

Two transgenic rodent gene mutation assays have been conducted using 1,3-D (mix of isomers) (Anonymous 24 (2018), Anonymous 25 (1997)). The rat study was conducted in accordance with OECD TG 488. While the study conducted in mice pre-dates the adoption of the 2010/2013 test guidelines, the design of the study was consistent with the critical elements of the guideline. For example, although the treatment period in the study was only 2 weeks (5 days/week), the animals were allowed an additional 17 days of expression time after the last exposure (i.e., mice were sacrificed on Day 27 from the start of the exposure) allowing ample time for the manifestation of mutations in the two tissues examined in the study (i.e.: liver and lung).

The transgenic Big Blue rodent assay is currently the only definitive means of detecting gene mutations *in vivo* (e.g.: base pair substitutions, frameshift mutations, insertions and deletions), it is viewed as the gold standard for assessing *in vivo* mutagenicity, especially in cases where the target organ for tumours is evaluated and is the recommended follow-on assay to address positive *in vitro* mutagenicity findings.

Both studies gave clear negative results and demonstrate that 1,3-D (mix of isomers) does not cause gene mutations in the liver, kidneys and lungs of transgenic rodents. It is therefore concluded that the substance does not have the potential to induce gene mutations in mammalian cells *in vivo.*

While no studies are available that have evaluated the potential of cis 1,3-D or trans 1,3-D to induce gene mutations in mammalian cells *in vivo,* the absence of positive findings in the studies conducted using 1,3-D (mix of isomers) indicates that the respective isomers lack mutagenic potential in this regard.

Mammalian chromosome aberrations assays

Four bone marrow micronucleus tests (3 in mice and 1 in rats) have been conducted with 1,3-D (mix of isomers) that have investigated the clastogenic potential of 1,3-D (mix of isomers) or the potential of the substance to alter the integrity or the function of the spindle apparatus (Anonymous 26 (1985), Shelby *et al. (*1993), Ghia *et al. (*1993), Kevekordes *et al*. (1996)).None of these tests were fully compliant with OECD TG 474 (2016), with the majority of deviations relating to the characterisation of the test substance used, a deficiency in the number of polychromatic erythrocytes (PCE) scored for micronuclei (MN) and a lack of reported information for individual animal MN PCE frequencies and historical control data (HCD).

Two of the studies, a bone marrow micronucleus assay in CD-1 (ICR) BR mice and a micronucleus assay in rat liver, bone marrow and spleen reported negative results for 1,3-D although in both cases increases in MN PCE were apparent, which failed to achieve the level of statistical significance defined by the authors as being indicative of a positive response.

Two of the studies reported positive results. The micronucleus test in the erythrocytes of NMRI mice reported an increase in the frequency of MN PCE in the bone marrow of female mice only, at a dose of 187 mg/kg bw/day 1,3-D (ratio cis:trans – 81:1). The study is considered to be unacceptable due to significant deviations from OECD 474 (2016) and a lack of any explanation for the clear difference in responses between male and female animals.

The study in B6C3F1 reported increases in MN PCE frequencies only after a single administration at a high dose of 150 mg/kg (approaching the LD_{50}) and only when sampled 48 h after dosing. At high exposures, the major detoxification pathway for 1,3-D (i.e.: glutathione conjugation) is overwhelmed and glutathione reserves are depleted. Under such conditions it is likely that the trace pathway of oxidation occurs resulting in an increase in reactive 1,3-D-epoxide. Under normal physiological conditions (such as those used in the *in vivo* transgenic rodent gene mutation test in somatic cells), the trace pathway results in little to no generation of reactive 1,3-D-epoxide and therefore no genotoxic effects. In the study, negative results were obtained at the 24 h sample time and at all dose levels tested using a 3-dose, 24 h sample regimen. Furthermore, the increased MN PCE frequency was shown to be within the laboratory's HCD and is therefore considered to be equivocal according to modern evaluation criteria (OECD 474, 2016).

The potential for trans 1,3-D to induce chromosome aberrations *in vivo* has been investigated in mice in a bone marrow micronucleus test conducted according to OECD TG 474 (adopted in 1997), following inhalation exposure to the substance at 150, 300 and 600 ppm ((Anonymous 47 (1999)). Adequate exposure of the target cells was demonstrated by evidence of cytotoxicity to the bone marrow cells by the test substance indicated by a statistically significant negative trend in the number of PCE per number of erythrocytes versus exposure level. Under the conditions of the study, trans 1,3-D did not produce an increased frequency of MN in PCE.

While an *in vivo* bone marrow micronucleus assay is not available for cis 1,3-D, the findings from the in vitro micronucleus assay indicate that this isomer is unlikely to be induce cytogenetic toxicity under physiological conditions. The weight of evidence drawn from across a number of *in vivo* micronucleus studies using 1,3-D (mix of isomers) containing cis 1,3-D at approximately 50 to 80% did not provide evidence of cytogenetic toxicity at doses that were not cytotoxic or at which the major detoxification pathways were over-whelmed.

DNA damage

Three studies in rats report the DNA damage effects of 1,3-D (mix of isomers) (Ghia *et al.* (1993), Kitchen and Brown (1994)). The studies: two alkaline elution assays and an UDS assay are not apical endpoints of mutagenicity, but rather are indicators of genotoxicity. In the liver UDS assay, 1,3-D (mix of isomers) gave negative results at a single time point (2-4 hr). In the alkaline elution assay 1,3-D (mix of isomers) did not induce DNA damage in lung, brain, or bone marrow. Positive results for DNA damage were however observed in the liver, stomach, and kidneys. Positive DNA damage was also reported in the rat liver in the alkaline elution assay.

Supporting information from experimental studies provides weight of evidence for the likely mechanism behind 1,3-D genotoxicity. Cytochrome P450-mediated metabolism has been shown to play a significant role in the DNA damage caused by 1,3-D. Furthermore, 1,3-D has been shown to cause significant GSH depletion within 1 hour of administration of a single 125 mg/kg dose. The observations of 1,3-D-induced GSH depletion *in vivo* provide further evidence that 1,3-D-mediated genotoxicity is directly linked to the role of glutathione in protecting cells from this toxicity.

Genotoxicity in vivo (germ cells)

The potential for 1,3-D (mix of isomers) to induce genotoxicity in germ cells *in vivo* has been investigated in two studies (Valencia *et al*. (1985), Anonymous 27 (1997)).

In a limited study conducted using Drosophila melanogaster in accordance with OECD TG 477 (deleted in 2014) with deviations and considered to be unacceptable, weakly positive/equivocal results were obtained in the induction of sex-linked recessive lethals and negative results were obtained in the test for reciprocal translocations.

Negative results were obtained in an acceptable dominant rodent lethal assay conducted using CD (SD-derived) rats in accordance with OECD TG 478.

While no studies are available that have evaluated the potential of cis 1,3-D or trans 1,3-D to induce genotoxicity in germ cells *in vivo,* the absence of positive findings in a reliable rodent lethal assay conducted

using 1,3-D (mix of isomers) indicates that the respective isomers lack the potential for genotoxicity in this regard.

Overall conclusion on genotoxicity

The available database of genotoxicity studies for 1,3-D (mix of isomers) enables the mutagenic potential of the substance to be thoroughly assessed and on this basis, a complete evaluation of genotoxicity can be made.

The dataset for evaluating the genotoxicity of 1,3-D *in vitro* and *in vivo* is extensive. While mixed results have been obtained in studies *in vitro*, many of the studies were confounded by the use of low purity or uncharacterised test material, often containing the known genotoxic stabilising agent, epichlorohydrin (use of this stabiliser was discontinued in 1983). Findings of structural and numerical chromosomal aberrations in other somatic cell assays, occurring at higher doses and close to the LD_{50} were mediated by over-whelmed detoxification systems and the formation of reactive epoxide species. Hence, *in vitro* studies that did identify genotoxic activity are not considered to be relevant to *in vivo* situations, since the addition of glutathione (GSH), which forms the major conjugated metabolite of 1,3-D leading to elimination, at normal physiological levels to the *in vitro* system was demonstrated to have a protective role.

Positive *in vitro* findings are not considered to be relevant to the hazard characterisation *in vivo* if robust *in vivo* studies demonstrate negative effects. Guideline *in vivo* genotoxicity studies conducted using 1,3-D test material not containing the epichlorohydrin stabiliser include: a mouse bone marrow micronucleus assay, dominant lethal rodent assay, a Big Blue transgenic mouse assay, a study of DNA adducts: 32P-post labelling assay and a Big Blue transgenic rat assay.

Two Big Blue transgenic rodent assays conducted using 1,3-D (mix of isomers) reported clear negative results. The transgenic Big Blue rodent assay is currently the only definitive means of detecting gene mutations *in vivo* (e.g.: base pair substitutions, frameshift mutations, insertions and deletions). This assay test system is therefore currently viewed as the gold standard for assessing *in vivo* mutagenicity, especially in cases where the target organ for tumours is evaluated and is the recommended follow-on assay to address positive *in vitro* mutagenicity findings.

The guideline *in vivo* studies have comprehensively assessed the genotoxic potential of 1,3-D. Endpoints evaluated in these studies include: the induction of clastogenicity/aneugenicity, the potential for the induction of mutations in male germ cells, the potential for induction of somatic cell mutations in rat liver and kidney as well as in mouse liver and lung tissues using the transgenic Big Blue assay and the potential for induction of DNA adducts in rat liver and in mouse lung tissues. The weight of evidence clearly indicates that 1,3-D does not present a genotoxic hazard *in vivo.*

In conclusion, the existing *in vivo* studies provide strong evidence that 1,3-D (mix of isomers) is not an *in vivo* mutagen or a germ cell mutagen and does not require classification for mutagenicity according to the CLP criteria. Available genotoxicity studies conducted using cis 1,3-D and trans 1,3-D provide comparable findings to the corresponding studies conducted using 1,3-D (mix of isomers). Based on a category approach, the grouping of these substances for classification in respect of this endpoint is therefore considered to be appropriate.

Carcinogenicity

The long-term toxicity and carcinogenicity of 1,3-D (mix of isomers) following oral administration or inhalation have been investigated in rats in three respective combined 2-year chronic toxicity/oncogenicity studies conducted according to OECD TG 453, considered to be acceptable with reservations (Anonymous 53 (1998), Anonymous 54 (1995), Anonymous 58 (1987)).

In an oral gavage study in which SD CR rats were dosed with 1,3-D at 0, 2, 10 and 25 mg/kg bw/day for 2 years, no carcinogenic effects were observed and the NOAEL for potential oncogenicity was determined to be 25 mg/kg bw/day, i.e.: the highest dose tested. The NOAEL for chronic toxicity was determined to be 2 mg/kg bw/day based on an increased incidence of and/or severity of hyperplasia and hyperkeratosis of the stratified squamous epithelium lining of the forestomach at higher doses.

In a dietary study in which Fischer 344 rats were dosed with 1,3-D at 0, 2.5, 12.5 and 25 mg/kg bw/day for 2 years, the NOAEL for potential oncogenicity was determined to be 2.5 mg/kg bw/day based on the increased

incidence of benign liver tumours (hepatocellular adenomas) in male rats at higher doses. The NOAEL for chronic toxicity was determined to be 2.5 mg/kg bw/day based on observations of basal cell hyperplasia of the non-glandular mucosa of the stomach in male and female rats at higher doses.

In an inhalation study in which Fischer 344 rats were exposed to vapours of 1,3-D at 0, 5, 20 or 60 ppm (0, 4.86, 19.44 or 58.32 mg/kg bw/day) for 2 years, no carcinogenic effects were observed. The NOAEL for chronic toxicity was determined to be 20 ppm (19.44 mg/kg bw/day) based on observations of reduced bodyweights and microscopic changes of the nasal epithelium (decreased thickness and erosion of the olfactory epithelium and submucosal fibrosis) at higher doses (in the interim report from the study from 12 months, the NOAEL for general toxicity was determined to be 20 ppm (17.74 mg/kg bw/day) based on observations of reduced bodyweights at higher doses).

In a carcinogenicity study conducted as part of US EPA National Toxicology Program, F344/N rats were dosed orally with 1,3-D (containing epichlorohydrin at 1% as a stabiliser) at 0, 25 or 50 mg/kg bw/day. The study is not considered to be acceptable due to deviations from OECD TG 451. While the NOAEL for carcinogenicity was determined to be 10.7 mg/kg bw/day (25 mg/kg bw/day x 3 day / 7 days) based on observations of forestomach (hyperplasia and squamous cell papilloma) and liver neoplasms at higher doses, these findings are considered to be attributable to epichlorohydrin, which has known mutagenic and carcinogenic properties, rather than to 1,3-D.

The long-term toxicity and carcinogenicity of 1,3-D following oral administration or inhalation have been investigated in mice in three respective 2-year chronic toxicity/oncogenicity studies conducted according to OECD TG 451 or 453 considered to be acceptable with reservations (Anonymous 52 (1997), Anonymous 57 (1995), Anonymous 56 (1987).

In an oral gavage study in which CD®-1 albino mice where dosed with 1,3-D at 0, 2, 10 or 25 mg/kg bw/day for at least 18 months, the NOAEL for oncogenicity was determined to be 10 mg/kg bw/day based on observations of benign submucosal mesenchymal tumours in the urinary bladder at higher doses and the NOAEL for chronic toxicity was determined to be 10 mg/kg bw/day based on observations of cellular hyperplasia and hypertrophy in the urinary bladder at higher doses.

In a dietary study in which B6C3F1 mice where dosed with 1,3-D at 0, 2.5, 25 or 50 mg/kg bw/day for 2 years, no signs of carcinogenicity were observed (the NOAEL for oncogenicity was determined to be 50 mg/kg bw/day i.e.: the highest dose tested). The NOAEL for chronic toxicity was determined to be 2.5 mg/kg bw/day based on observations of reduced bodyweights and food consumption at higher doses.

In an inhalation study in which B6C3F1 mice were exposed to vapours of 1,3-D at 0, 5, 20 or 60 ppm (0, 8.42, 33.70 or 101.09 mg/kg bw/day) for 2 years, the NOAEL for oncogenicity was determined to be 20 ppm (33.70 mg/kg bw/day) based on an increased incidence of benign lung tumours at higher doses. The NOAEL for chronic toxicity was determined to be 5 ppm (8.42 mg/kg bw/day) based on observations of lesions of the urinary bladder and nasal mucosa, hyperplasia of the epithelial of the non-glandular stomach, decreased bodyweight and modified organ weight at higher doses (in the interim report from the study from 12 months, the NOAEL was determined to be 20 ppm (90.8 mg/kg bw/day) based on observations of hyperplasia and hypertrophy of the respiratory epithelium of the nasal at higher doses).

In a carcinogenicity study conducted as part of US EPA National Toxicology Program, B6C3F1 mice were dosed orally with 1,3-D (containing epichlorohydrin at 1% as a stabiliser) at 0, 50 or 100 mg/kg bw/day. The study is not considered to be acceptable due to deviations from OECD TG 451. While the LOAEL for carcinogenicity was determined to be 21.4 mg/kg bw/day (50 mg/kg bw/day x 3 day / 7 days) based on observations of urinary bladder and lung neoplasms at the lowest dose tested, these findings are considered to be attributable to epichlorohydrin, which has known mutagenic and carcinogenic properties, rather than to 1,3-D.

No studies are available that have specifically investigated the long-term, chronic toxicity or the carcinogenicity of cis 1,3-d or trans 1,3-D. While no studies are available, the carcinogenic potential of 1,3-D (mix of isomers) has been comprehensively evaluated in six reliable bioassays indicating the respective isomers are unlikely to have any carcinogenic potential.

Conclusion on carcinogenicity

An extensive database of studies is available to assess the carcinogenic potential of 1,3-D, comprising six qualified and well-conducted cancer bioassays conducted in rats and in mice covering multiple routes of exposure i.e.: dietary, oral gavage and inhalation. Oral route carcinogenicity studies conducted in F344 rats and in B6C3F1 mice as part of the National Toxicology Program (NTP, 1985) are considered to be compromised due to the presence of the stabiliser, epichlorohydrin (with known mutagenic and carcinogenic properties) at ~1% in the test material.

The four oral cancer bioassays are considered to be adequate and appropriate for assessing the tumorigenicity of 1,3-D on the basis that: the test material in each case included epoxidized soyabean oil (ESO) as a stabiliser instead of epichlorohydrin, representative of current manufactured product; tumorigenicity was evaluated in both rat and mouse rodent species and the bioassays were conducted under GLP and were guideline-compliant. In these studies, one tumour type only (benign liver tumour) in a single animal species and strain (F344 rats) was identified, with significantly increased liver tumours occurring at high doses exceeding the maximum tolerated dose (MTD) and with corresponding decreased body weight of ~20%. The liver tumours were benign, late in onset and lacked preneoplastic liver lesions. Evaluations from other rodent studies provide evidence that the liver is not a target organ. On the basis of this evidence, 1,3-D is not tumorigenic to the liver via the oral route at doses below the MTD i.e.: < 12.5 mg/kg bw/day.

Findings from two inhalation bioassays indicate that prolonged inhalation exposure of male B6C3F1 mice to a high concentration of 1,3-D (60 ppm) is associated with an increased incidence of portal-of-entry, benign lung adenomas. Comparable findings were not observed in female mice or in rats. A toxicokinetic study conducted in male B6C3F1 mice exposed via nose-only inhalation to 1,3-D at 0, 10, 20, 40, 60, 90 and 120 ppm has demonstrated that the tumorigenic dose of 60 ppm associated with the incidence of benign lung tumours exceeds the kinetically derived maximum tolerable dose (KMD). This dose is ~600-times higher than the highest 1,3-D concentrations measured in ambient air and is therefore of questionable relevance to humans.

On the basis of this evidence, 1,3-D is not a tumorigen to the lungs via the inhalation route at exposures below the KMD (i.e.: 60 ppm). The lung is not considered to be a target organ as no lung tumours were observed in any of the other cancer bioassays (oral or inhalation routes).

Based on the evaluation of the dataset of carcinogenic studies, the following conclusion are drawn:

- A statistically significant increase in incidence of benign liver tumours (adenomas) was observed in male Fischer 344 rats only (comparable findings were not observed in female Fischer 344 rats or in the chronic studies with mice) at a dose level which also caused significant reductions in body weight and body weight gain and is considered to exceed the MTD for the chronic administration of 1,3-D in rats. The observed increase in incidence is only just outside the historical control range for this tumour type and there is no evidence for reduced latency or progression to malignancy.
- A statistically significant increase in incidence of benign lung tumours (bronchioloalveolar adenomas) was observed in male B6C3F1 mice at 60 ppm (22/50 vs. 9/50 in controls). Bronchioloalveolar adenomas are a commonly observed spontaneous tumour type in this strain of mice and more commonly observed in males. The incidence of 22/50 is only just outside the background incidence which goes up to 18/50. There was no increase in lung tumours in female B6C3F1 mice, nor in Fischer 344 rats following chronic inhalation exposure to 1,3-D. The increased incidence in males was only observed at an exposure level that exceeds the KMD for 1,3-D in mice following inhalation exposure. There is no reduction in the tumour latency, adverse effects on survival or evidence of progression to malignancy.
- The slightly increased incidence of benign submucosal mesenchymal tumours in the urinary bladder of female CD-1 mice at 25 mg/kg bw/day (3/65) compared to controls (0/65) lacked statistical significance and were considered to be a secondary response to chronic irritation, representing a benign proliferative lesion indicating minimal or equivocal neoplastic activity. There was no increase of this tumour type in male CD-1 mice, nor in male or female B6C3F1 mice tested up to a higher dose level of 50 mg/kg/day, nor in Fischer 344 rats.

In conclusion, the existing dataset of carcinogenicity studies conducted using 1,3-D (mix of isomers) indicates that the compound does not require classification for carcinogenicity according to the CLP criteria. The available experimental data for cis 1,3-D and trans 1,3-D addressing toxicokinetics, acute toxicity, repeated dose toxicity and genotoxicity provide evidence that these isomers have a comparative toxicological profile.

Hence, the respective isomers are not expected to have carcinogenic potential and the grouping of these substances in respect of this endpoint, with the resulting non-classification for the group overall is considered to be appropriate.

Table A3: 1,3-dichloropropene (mixture of cis and trans isomers), (Z)-1,3-dichloropropene (cis isomer) and (E)-1,3-dichloropropene (trans isomer): toxicological data matrix

Endpoint	**1,3-dichloroproprene (mixture of cis and trans isomers**	**(Z)-1,3-dichloroproprene (cis isomer)**	**(E)-1,3-dichloropropene (trans isomer)**
	EC No. 208-826-5	**EC No. 233-195-8**	**EC No. 431-460-4**
	Cas No. 542-75-6	**Cas No. 10061-01-5**	**Cas No. 10061-02-6**
Acute oral toxicity	[1,3-D; 97.2%Telone II 86/3293] Acute oral toxicity; OECD TG 401 **LD_{50} (SD CFY rats) = 150 (110-170) mg/kg bw** Anonymous 8 (1986)	[cis 1,3-D; purity not stated] Acute oral toxicity; OECD TG 401 **LD_{50} (SD rats) = 121 (95% C.I, = 107-137) mg/kg bw** Anonymous 17 (1988)	[trans 1,3-D; 96.7%] Acute oral toxicity EEC Method B1 **LD_{50} (Fischer F344 rats) = 94 mg/kg bw** Anonymous 18 (1988)
	[Telone II: 51.4% cis/45.8% trans] Acute oral toxicity (non guideline) **LD_{50} (CD SD rats) = 424.3 mg/kg (rodent chow slurry)** Anonymous 9 (2006)	[cis 1,3-D; 95.45%] Acute oral toxicity; OECD TG 401 **LD_{50} (Fischer F344 rats) = 85 (95% C.I. = 76-96) mg/kg bw** Anonymous 23 (1989)	
Acute dermal toxicity	[Telone II] Acute dermal toxicity; OECD TG 402 **LD_{50}(SD CFY rats) = 1200 mg/kg bw** Anonymous 10 (1986)	[cis 1,3-D; purity not stated] Acute dermal toxicity; OECD TG 402 **LD_{50} (SD CFY rats) = 794 (95% C.I = 669-942) mg/kg bw** Anonymous 19 (1988)	[trans 1,3-D; 96.7%] Acute dermal toxicity; EEC Method B3 **LD_{50} (Fischer F344 rats) = 1575 (95% C.I. = 1163-3111) mg/kg bw** Anonymous 18 (1988)
	[AGR 233011; 52.63% cis/44.91 % trans] Acute dermal toxicity EPA Guideline 81-2 **LD_{50}(NZW rabbits) = 333 mg/kg bw/day** Anonymous 11 (1987)	[cis 1,3-D; 95.45%] Acute dermal toxicity (OECD TG 402) **LD_{50} (Fischer F344 rats) = 1090 (95% C.I = 901-1403) mg/kg]** Anonymous 23 (1989)	
Acute inhalation toxicity	[Telone II; 98.4% 1,3-D] Acute inhalation toxicity (whole body); OECD TG 403 **4-hr LC_{50} (Wistar rats) = 2.70-3.07 mg/L** Anonymous 12 (1987)	[cis 1,3-D; 95.6%] Acute inhalation toxicity; EPA Guideline 81-3 **4-hr LC_{50} (rats) = 670 ppm (males); 744 ppm (female)** Anonymous 20 (1990).	[trans 1,3-D; 95.4%] Acute inhalation toxicity (head only); OECD TG 403 **4-hr LC_{50} (Crl:CD(SD)BR rats) = 5.098 (4.54-6.092) mg/L (1123 ppm; 1000-1342 ppm)**

Endpoint	1,3-dichloroproprene (mixture of cis and trans isomers	(Z)-1,3-dichloroproprene (cis isomer)	(E)-1,3-dichloropropene (trans isomer)
	EC No. 208-826-5	**EC No. 233-195-8**	**EC No. 431-460-4**
	Cas No. 542-75-6	**Cas No. 10061-01-5**	**Cas No. 10061-02-6**
	[Telone II; AGR 233011; 52.8% cis; 44.9% trans] Acute inhalation toxicity (whole body); OECD TG 403/EPA guidelines **4-hr LC_{50} (Fisher 344 rats) = 3.88-4.70 mg/L** Anonymous 13 (1987).		Anonymous 21 (1989).
Skin irritation/corrosion	[AGR 233011; 52.63% cis; 44.91 % trans] Skin irritation study; EPA 81-5 **Irritating (NZW rabbits)** Anonymous 14 (1987).	[cis 1,3-D; 95.45%] Acute dermal irritation; OECD TG 404 skin irritant] Anonymous 23 (1989)	[trans 1,3-D; 96.7%] Skin irritation study; EEC Method B4 **Irritation (NZW rabbits)** - not exceeding well-defined erythema and slight oedema: resolved within 21 days Anonymous 18 (1988)
Eye irritation	[AGR 233011; 52.63% cis; 44.91% trans] Eye irritation study; EPA 81-4 **Irritating to eyes (NZW rabbits)** Anonymous 15 (1987)	[cis 1,3-D; 95.45%] Isolated Eye Test (ex vivo study) **Serious eye irritation (NZW rabbits)** Anonymous 23 (1989)	[trans 1,3-D; 96.7%] Eye irritation study; EEC Method B5 **Not irritating (NZW rabbits)** Anonymous 18 (1988).
Skin sensitization	[AGR 233011; 52.63% cis; 44.91% trans] Buehler test; OECD TG 406 **Skin sensitizer (Guinea pigs)** Anonymous 16 (1987)	Buehler test; OECD TG 406. **Skin sensitizer (Guinea Pigs)** Anonymous 22 (1988). [cis 1,3-D; 95.45%] Guinea pig maximisation test; OECD TG 406 **Skin sensitiser (Guinea Pigs)** Anonymous 23 (1989)	[trans 1,3-D; 96.7%] Skin sensitization test (Guinea pig maximization test) ; EEC Method B6 **Skin sensitizer (Guinea pigs)** Anonymous 18 (1988).
Short-term repeated dose toxicity – up to 28 days	Palatability and 2-week dietary probe studies in mice, rats and dogs (DAR, 2018) 4-week vapour inhalation probe study in dogs (DAR, 2018)	14-day inhalation toxicity study in Fischer 344 rats Dosing: 0, 10, 60 and 150 ppm vapours **NOAEL: 60 ppm** (m/f): based on changes in body weight, non-protein sulfhydryl levels at 1-hour in liver, kidney and lung and histopathological changes of moderate severity in the respiratory and olfactory mucosa in the nasal cavity in	[trans 1,3-D 96.7%] 14-day inhalation toxicity study in Wistar rats OECD TG 412 Dosing: 0, 30, 100, 300 ppm DRF study proposed doses of 0, 10, 30 and 90 ppm for a 90-day inhalation toxicity study. Anonymous 50 (1999)

Endpoint	1,3-dichloroproprene (mixture of cis and trans isomers	(Z)-1,3-dichloroproprene (cis isomer)	(E)-1,3-dichloropropene (trans isomer)
	EC No. 208-826-5	**EC No. 233-195-8**	**EC No. 431-460-4**
	Cas No. 542-75-6	**Cas No. 10061-01-5**	**Cas No. 10061-02-6**
		male and female rats exposed to 150 ppm **LOAEL: 150 ppm** (m/f): based on changes in body weight, non-protein sulfhydryl levels at 1-hour in liver, kidney and lung and histopathological changes of moderate severity in the respiratory and olfactory mucosa in the nasal cavity in male and female rats exposed to 150 ppm Anonymous 48 (1990)	
Short-term repeated dose toxicity – sub-chronic 90 - day	13-week dietary + 4-week recovery studies in rats and mice; 1-year dietary study in dogs (DAR, 2018) Sub-chronic 90-day inhalation study in rats and mice (DAR, 2018)	90-day sub-chronic inhalation toxicity study in Fischer 344 rats OECD TG 413 Dosing: 0, 10, 30 and 90 ppm vapours **NOEL: 30 ppm (m/f)** – based on decreased bodyweight of males and hyperplasia of the nasal and respiratory epithelia of males and females exposed to 90 ppm **LOEL: 90 ppm** (m/f) - based on decreased body weight of males and hyperplasia of the nasal and respiratory epithelia of males and females Anonymous 49 (1991)	[trans 1,3-D; 96.7%] 90-day sub-chronic inhalation study in Wistar (Crl:(WI)WU BR) rats OECD TG 413 Dosing: 0, 10, 30 and 90 ppm **NOAEL: 8.9 ppm** – based on decreased bodyweight gain in animals in the mid and high dose groups. Anonymous 51 (1999)
In vitro genotoxicity (bacterial cells)	[1,3-D 96.1% (49.8% cis/46.3% trans) Bacterial reverse mutation assay Pre-guideline (not acceptable) **Positive** (In TA1535 and TA100 was positive (>2-fold increase in revertants), and in TA98 and G46, weak positive (±S9, 1.5-2-fold increase in revertants). Anonymous 41 (1978)	[cis 1,3-D >99%] Bacterial reverse mutation assay Pre-guideline (not acceptable) **Positive** in TA1535, TA100 and TA98 (±S9) Anonymous 28 (1978)	No studies available

Endpoint	1,3-dichloroproprene (mixture of cis and trans isomers	(Z)-1,3-dichloroproprene (cis isomer)	(E)-1,3-dichloropropene (trans isomer)
	EC No. 208-826-5	**EC No. 233-195-8**	**EC No. 431-460-4**
	Cas No. 542-75-6	**Cas No. 10061-01-5**	**Cas No. 10061-02-6**
	[1,3-D 95% (51.3$ cis; 43.7% trans) Bacterial reverse mutation assay Pre-guideline (not acceptable) **Positive in TA1535, TA100 (±S9).** Anonymous 28 (1978)	Bacterial reverse mutation assay; guideline not stated: supplemental **Positive:** in TA100 and TA1535 Anonymous 42 (1978).	
	[1,3-D 97.6%; 51.7% cis; 45.9% trans)] Bacterial reverse mutation assay; OECD TG 471 (not acceptable) **Negative** Anonymous 32 (1996)	[cis 1,3-D 99%] Bacterial reverse mutation assay; guideline not states: supplemental **Positive:** in TA1535, TA98 and T100 +/- S9 mix Anonymous 43 (1978).	
	[1,3-D 97.6% (49.9% cis; 47.7% trans)] Bacterial reverse mutation assay Non-guideline (not acceptable) **Negative** Anonymous 34 (1997)	[Cis 1,3-D 94.51-97.51%] Bacterial reverse mutation assay; OECD TG 471 **Positive**: in WP2 uvrA pkm101, TA 1535, TA100 +/- S9 mix Anonymous 44 (1990).	
	[1,3-D 96% (49.3% cis; 46.7% trans) Bacterial reverse mutation assay; OECD TG 472; acceptable **Positive:** TA1535 and TA100 -S9. TA 1535 +S9 (Mutagenicity decreased in the presence of S9) Anonymous 35 (1999)		
	[1,3-D 99.64% (51.36% cis; 48.28% trans)] Bacterial reverse mutation assay; guideline not stated: supplemental **Positive:** TA1535 and TA100 strains (+/- purified rat liver microsomes) Anonymous 33 (2009)		
	[1,3-D 99.64%; mix?]		

Endpoint	1,3-dichloroproprene (mixture of cis and trans isomers	(Z)-1,3-dichloroproprene (cis isomer)	(E)-1,3-dichloropropene (trans isomer)
	EC No. 208-826-5	**EC No. 233-195-8**	**EC No. 431-460-4**
	Cas No. 542-75-6	**Cas No. 10061-01-5**	**Cas No. 10061-02-6**
	Bacterial reverse mutation assay; non guideline: supplemental **Positive** for TA1535 (-M) **For all 3 species:** **Positive** for TA1535 (+M) **Negative** for TA1535 (+S100, +GSH) **Negative** for TA1535 (+M, +S100, +GSH) Anonymous 31 (2004)		
	[1,3-D (49.4% cis; 42.5% trans] Bacterial reverse mutation assay; OECD TG 471: supplemental **Mouse Urine -** Negative for TA 100 and TA98 ± S9. **N-acetylcysteine conjugate** Positive for TA100 (-S9) (single experiment) **Cysteine conjugate -** Positive for TA100 (-S9) & TA98 (-S9). Negative in the presence of a kidney homogenate. Poor substrate of hepatic and renal beta-lyase *in vitro.* **sulfoxide/sulfone conjugate -** Positive for TA100 (-S9) & TA98 (-S9) **thioglycolic acid conjugate** - Positive for TA100 (-S9) **Disulfide –** Negative (-S9) (single experiment) Anonymous 39 (1989)		
In vitro gene mutation (mammalian cells)	[1,3-D 92.1% (48.9% cis; 43.2% trans)] In vitro mammalian cell gene mutation assay HPRT test ($CHO\text{-}K_1\text{-}BH_4$) OECD TG 476: Supplemental **Negative** Anonymous 36 (1986)	No studies available	No studies available
In vitro chromosome aberration (mammalian cells)	[1,3-D 95.3%] Chromosome aberration assay (CHL cells) OECD TG 473: Supplemental	[cis 1,3-D 94.51-97.51%] Chromosome aberration assay (CHO) EPA Guideline 84-2; OECD TG 473	[trans 1,3-D 96.7%] Chromosome aberration assay (CHO cells) EPA Pesticide Assessment Guideline 84-2: Supplemental

Endpoint	1,3-dichloroproprene (mixture of cis and trans isomers	(Z)-1,3-dichloroproprene (cis isomer)	(E)-1,3-dichloropropene (trans isomer)
	EC No. 208-826-5	**EC No. 233-195-8**	**EC No. 431-460-4**
	Cas No. 542-75-6	**Cas No. 10061-01-5**	**Cas No. 10061-02-6**
	Positive: Clastogenic +/- S9, aneugenic -S9 mix after 48 hrs Anonymous 38 (1988)	**Positive:** +S9 mix; negative -S9 (chromosome damage reduced by GSH) Anonymous 45 (1991).	**Positive**: clastogenic +S9 mix; negative -S9 mix Anonymous 46 (1989)
In vitro DNA damage/DNA binding (mammalian cells)	[1,3-D 95% (cis:trans – 81:1 ratio)] Sister-chromatic exchange (SCE) test with human lymphocytes OECD TG 479: Unacceptable **Positive** Kevekordes *et al.* (1996)	No studies available	No studies available
	[1,3-D 92.1% (49.5% cis; 42.6% trans) UDS assay in primary hepatocytes: CD Fischer 344 rats; OECD TG 482: Supplemental **Negative** Anonymous 37 (1985)		
	[1,3-D >99% (cis/trans – 50/50)] In vitro DNA binding (adduct) assay OECD TG 482: Supplemental **Negative** Anonymous 40 (1997)		
In vivo genotoxicity – somatic cells: Gene mutation	**[**1,3-D 33.7% cis/trans mix – microencapsulated in 80% starch and 20% sucrose)] Gene mutation assay at the *cII* Locus in male Big Blue transgenic F344 rats (oral) OECD TG 488, Acceptable **Negative:** No mutations induced in the *cII* gene in the liver and kidneys of male Big Blue rats Anonymous 24 (2018)		
	[1,3-D (49.3% cis; 46.7% trans)]		

Endpoint	1,3-dichloroproprene (mixture of cis and trans isomers	(Z)-1,3-dichloroproprene (cis isomer)	(E)-1,3-dichloropropene (trans isomer)
	EC No. 208-826-5	**EC No. 233-195-8**	**EC No. 431-460-4**
	Cas No. 542-75-6	**Cas No. 10061-01-5**	**Cas No. 10061-02-6**
	Gene mutation assay at the lacL target gene of transgenic Big Blue B6C3F1 male mice Pre-guideline but similar to OECD TG 488 **Negative:** No mutations induced in the lacL gene in the liver or the lung tissue of male Big Blue mice Anonymous 25 (1997)		
In vivo genotoxicity – somatic cells: Chromosome aberration	[1,3-D 92.1% (49.5% cis, 42.6% trans)] Bone-marrow micronucleus assay in mice (CD-1 (ICR) BR) (oral gavage) OECD TG 474: Supplementary **Negative** Anonymous 26 (1985)	No studies available	[trans 1,3-D 96.7%] Micronucleus test in mouse erythrocytes OECD TG 474: Acceptable **Negative** Anonymous 47 (1999).
	[1,3-D purity not specified] Bone marrow micronucleus assay in mice (B6C3F1) (i.p. route) OECD TG 474: Supplementary **Equivocal** (when tested a=>150 mg/kg bw) Shelby *et al. (*1993)		
	[1,3-D; 98%; cis/trans 50/50] Micronucleus test in rat liver, bone marrow and spleen OECD TG 474: Supplemental **Negative** Ghia *et al. (*1993)		
	[1,3-D; 95%, cis/trans 81:1] Micronucleus test in mouse erythrocytes OECD TG 479: Unacceptable **Positive** (in females but not in males) Kevekordes *et al*. (1996)		
In vivo genotoxicity – somatic	[1,3-D; 98%; cis/trans 50/50] DNA damage OECD TG 474: Supplemental		

Endpoint	1,3-dichloroproprene (mixture of cis and trans isomers	(Z)-1,3-dichloroproprene (cis isomer)	(E)-1,3-dichloropropene (trans isomer)
	EC No. 208-826-5	**EC No. 233-195-8**	**EC No. 431-460-4**
	Cas No. 542-75-6	**Cas No. 10061-01-5**	**Cas No. 10061-02-6**
cells: DNA damage	**Positive** (only in gastric mucosa, kidney and liver] Ghia *et al.* (1993)		
	[1,3-D; 98%; cis/trans 50/50] UDS in hepatocytes OECD TG 474: Acceptable **Negative** Ghia *et al.* (1993)		
	[1,3-D; purity not reported] DNA damage in female rat liver (alkaline elution assay) Non guideline: Unacceptable **Positive** (DNA damage at 94 mg/kg Kitchen and Brown (1994)		
In vivo genotoxicity – germ cells	[1,3-D 95.5%] Drosophila melanogaster - sex-linked recessive lethals (SLRL) induction, and reciprocal translocations (RT) OECD TG 477: Unacceptable **SLRL – Weak positive/equivocal** **RT – Negative** Valencia *et al.* (1985)	No studies available	No studies available
	[1,3-D: 96% (49.3% cis, 46.7 % trans); 96.5% (49.87% cis, 46.59% trans)] Dominant lethal assay in CD SD rat OECD TG 478: Acceptable **Negative** Anonymous 27 (1997)		
Long-term chronic toxicity/carcinogenicity	[1,3 D – DD-92] 2-year chronic toxicity/oncogenicity study in SD CR rats (oral gavage) OECD TG 453: Acceptable with reservations Dosing: 0, 2, 10 and 25 mg/kg bw/dd **Not carcinogenic** **NOAEL (chronic) = 2 mg/kg bw/d** **NOAEL (oncogenic) = 25 mg/kg bw/d**	No studies available	No studies available

Endpoint	1,3-dichloroproprene (mixture of cis and trans isomers	(Z)-1,3-dichloroproprene (cis isomer)	(E)-1,3-dichloropropene (trans isomer)
	EC No. 208-826-5	**EC No. 233-195-8**	**EC No. 431-460-4**
	Cas No. 542-75-6	**Cas No. 10061-01-5**	**Cas No. 10061-02-6**
	Anonymous 53 (1998)		
	[1,3-D 96% (50.7% cis, 45.1% trans) Telone II] 2-year chronic toxicity/oncogenicity study in Fischer 344 rats (dietary) OECD TG 453: Acceptable with reservations Dosing: 0, 2.5, 12.5 and 25 mg/kg bw/d **Increased incidence of benign liver tumours** **NOAEL (chronic) = 2.5 mg/kg bw/d** **NOAEL (oncogenic) = 2.5 mg/kg bw/d** Anonymous 54 (1995)		
	[1,3-D 92.1% 49.5% cis; 42.6% trans Telone II] 2-year chronic toxicity/oncogenicity study in Fischer 344 albino rats (inhalation) OECD TG 453: Acceptable with reservations Dosing: 0, 5 20, 60 ppm (0, 4.86, 19.44, 58.32 mg/kg bw/d) **Not carcinogenic** **NOAEL (chronic) = 20 ppm (19.44 mg/kg bw/d)** Anonymous 56 (1987) Interim report 12 months: Dosing: 0, 5, 20, 60 ppm (0, 4.43, 17.74 and 53.22 mg/kg bw/d **NOAEL (chronic) = 20 ppm (17.74 mg/kg bw/d** Anonymous 55 (1985)		
	[Telone II + 1% epichlorohydrin] NTP carcinogenicity study in F344/N rats OECD TG 451: Not acceptable NOAEL (carcinogenicity) = 10.7 mg/kg bw		

Endpoint	1,3-dichloroproprene (mixture of cis and trans isomers	(Z)-1,3-dichloroproprene (cis isomer)	(E)-1,3-dichloropropene (trans isomer)
	EC No. 208-826-5	**EC No. 233-195-8**	**EC No. 431-460-4**
	Cas No. 542-75-6	**Cas No. 10061-01-5**	**Cas No. 10061-02-6**
	TOXICOLOGICAL REVIEW OF 1,3-DICHLOROPROPENE (CAS No. 542-75-6) - In Support of Summary Information on the Integrated Risk Information System (IRIS), *May 2000* - EPA/635/R-00/001		
	[1,3-D DD-92] Oncogenicity study in CD-1 mice (oral gavage) OECD TG 451: Acceptable Dosing: 0, 2, 10 and 25 mg/kg bw/d **Benign submucosal mesenchymal tumours in the urinary bladder** **NOAEL (chronic) = 10 mg/kg bw/d** **NOAEL (oncogenic) = 10 mg/kg bw/d** Anonymous 52 (1997)		
	[1,3-D 95.8% (50.5% cis, 45.1 % trans)] 2-year chronic toxicity/oncogenicity study in B6C3F1 mice (dietary) OECD TG 453: Acceptable with reservations Dosing: 0, 2.5, 25 and 50 mg/kg bw **Not carcinogenic** **NOAEL (chronic) = 2.5 mg/kg bw/d** **NOAEL (oncogenic) = 50 mg/kg bw/d** Anonymous 57 (1995)		
	[1,3-D 92.1% 49.5% cis; 42.6% trans Telone II] 2-year chronic toxicity/oncogenicity study in B6C3F1 mice (inhalation) OECD TG 453: Acceptable with reservations Dosing: 0, 5 20, 60 ppm (0, 8.42, 33.7, 101.09 mg/kg bw/d) **NOAEL (oncogenic) = 20 ppm (33.70 mg/kg bw/d)**		

Endpoint	1,3-dichloroproprene (mixture of cis and trans isomers	(Z)-1,3-dichloroproprene (cis isomer)	(E)-1,3-dichloropropene (trans isomer)
	EC No. 208-826-5	**EC No. 233-195-8**	**EC No. 431-460-4**
	Cas No. 542-75-6	**Cas No. 10061-01-5**	**Cas No. 10061-02-6**
	NOAEL (chronic) = 5 ppm (8.42 mg/kg bw/d) Anonymous 56 (1987) Interim report 12 months: Dosing: 0, 5, 20, 60 ppm (0, 22.7, 90.8, 272.4 mg/kg bw/d **NOAEL (chronic) = 20 ppm (90.8 mg/kg bw/d** Anonymous 55 (1985)		
	[Telone II + 1% epichlorohydrin] NTP carcinogenicity study in B6C3F1 mice OECD TG 451: Not acceptable LOAEL (carcinogenicity) = 21.4 mg/kg bw TOXICOLOGICAL REVIEW OF 1,3-DICHLOROPROPENE (CAS No. 542-75-6) - In Support of Summary Information on the Integrated Risk Information System (IRIS), *May 2000* - EPA/635/R-00/001		
Reproductive toxicity	Hazard class not assessed in the dossier	Hazard class not assessed in the dossier	Hazard class not assessed in the dossier
Developmental toxicity	Hazard class not assessed in the dossier	Hazard class not assessed in the dossier	Hazard class not assessed in the dossier

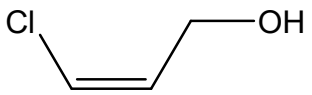
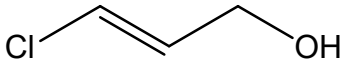
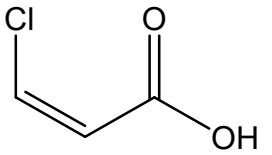
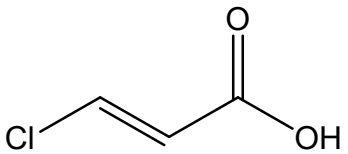
Environmental Hazards

One test is available to assess the ready biodegradability of 1,3-dichloropropene (Telone II). Based on this study 1,3-D is classified as not readily biodegradable. There are no separate studies available for the two isomers; due to the lack of observed degradation, it can be inferred that both isomers are not readily biodegradable.

The hydrolytic degradation of (Z)-1,3-dichloropropene (cis isomer) and (E)-1,3-dichloropropene (trans isomer) was investigated in two studies. These studies were conducted under GLP standards according to internationally accepted guidelines (e.g., OECD). Tests were conducted in sterile, buffered solutions at three pHs (4, 7, and 9). At pH 7, 1,3-D hydrolysed under sterile conditions with a single first order DT50 at 25°C of 2.69 days (Z)-1,3-dichloropropene (cis isomer) and 4.75 days (E)-1,3-dichloropropene (trans isomer). The degradation appeared to be independent of pH. Based on these two studies, it can be concluded that 1,3-dichloropropene is rapidly degraded by hydrolysis environmental pH conditions.

The below listed metabolites are formed under environmental conditions and have been observed in the listed compartments. As with the parent compounds these are in isomeric forms. Both isomeric forms have been studied extensively as metabolites under the plant protection active substance process.

Table A4: Overview of metabolites

Code Number (Synonyms)	Description	Compound found in:	Structure
CAA, CAAL	Cis-and trans 3-chloroallyl alcohol	Soil, water	Cis (*Z*) 3-chloroallyl alcohol  Trans (*E*) 3-chloroallyl alcohol 
CAC, CAAc	Cis and trans 3-chloroacrylic acid	Soil, water	Cis (*Z*) 3-chloroacrylic acid  Trans (*E*) 3-chloroacrylic acid 

A read across matrix has been prepared for acute environmental endpoints below, comparisons and conclusions. For full study summaries, references and acceptability conclusions see section 11.5 of the CLH report. Only studies considered acceptable have been compared below.

Table A5: Data matrix for environmental endpoints

Trophic level	1,3-dichloropropene (Mixed isomer)	(Z)-1,3-dichloropropene (Cis isomer)	(E)-1,3-dichloropropene (Trans isomer)
Plants	*Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata* also formerly known as *Selenastrum capricornutum*) 96-hour ErC50: 5.45 mg as/L Anonymous 102, 2013	*Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) 24-72h ErC50 = 3.1mg/L Anonymous 87, 1989	N/A
Invertebrates	*Daphnia magna* 48-hour EC50 =1.83 mg/L Draft Assessment Report, Spain, Volume 3 - B.9 (AS), July 2018 CA 8.2.4.1/3 Eastern oyster (*Crassotrea virginica*) 96-hour EC50 0.64 mg as/L (based on inhibition of shell growths) Anonymous 89, 1999	*Daphnia magna* 48h EC50 = 1.4 mg/L Anonymous 87, 1989	N/A
Fish	Rainbow trout, *Oncorhynchus mykiss* 96-h LC50: 2.78 mg/L Draft Assessment Report, Spain, Volume 3 - B.9 (AS), July 2018CA 8.2.1/1 Anonymous 91, 2001 Sheepshead minnow, *Cyprinodon variegatus* 96-h LC50: 0.87 mg/L Anonymous 92, 1999	Rainbow trout (*Salmo gairdneri*) 96h LC50 = 1.6 mg/L Anonymous 87, 1989 Sheepshead minnow results **not reliable**	N/A **Reliable with restriction.** Sheepshead minnow, *Cyprinodon variegatus* 0.290 ppm (0.29 mg/L) Anonymous 88, 1978

Acute study comparison by trophic level

Plants

There is no data available for (E)-1,3-dichloropropene (Trans isomer). The algae species *Raphidocelis subcapitata* was tested using both 1,3-dichloropropene and (Z)-1,3-dichloropropene (Cis isomer) with 96h and 72h ErC50 values of 5.45 mg/L and 3.1 mg/L respectively. The results for the mixture of isomers and the (Z)-

1,3-dichloropropene (Cis) isomer are in the same order of magnitude, this infers that the (E)-1,3-dichloropropene (Trans) isomer will exhibit toxicity in the same order of magnitude.

Invertebrates

There is no data available for (E)-1,3-dichloropropene (Trans isomer). The invertebrate *Daphnia magna* was tested using both 1,3-dichloropropene and (Z)-1,3-dichloropropene (Cis isomer) with 48h and 72h EC50 values of 1.83 mg/L and 1.4 mg/L respectively. The results for the mixture of isomers and the (Z)-1,3-dichloropropene (Cis) isomer are in the same order of magnitude, this infers that the (E)-1,3-dichloropropene (Trans) isomer will exhibit toxicity in the same order of magnitude.

Fish

The same fish species, Sheepshead minnow *Cyprinodon variegatus*, has been tested in for both 1,3-dichloropropene (Mixed isomer) and (E)-1,3-dichloropropene (Trans isomer) with 96h LC50 values of 0.87 mg/L and 0.29 mg/L respectively. The results for 3-dichloropropene (Mixed isomer) and (E)-1,3-dichloropropene (Trans isomer) are the same order of magnitude. Both of these results would lead to the classification of Very toxic to aquatic life (H400).

Similarly, the Rainbow trout has been tested for both 1,3-dichloropropene (Mixed isomer) and (Z)-1,3-dichloropropene (Cis isomer) and the results are the same order of magnitude. There is no indication that the isomers will differ significantly in toxicity with results being consistently within the same order of magnitude for each species.

Acute comparison conclusion

In conclusion, the evaluation of available experimental data on 1,3-dichloropropene (mixture of isomers), (Z)-1,3-dichloropropene (cis isomer) and (E)-1,3-dichloropropene (trans isomer) indicates that these substances have a comparable profile with respect to potential for acute aquatic hazard. These substances respectively meet the CLP criteria for classification of Very toxic to aquatic life (H400). Based on a category approach, the grouping of these substances for classification in respect of this endpoint is therefore considered to be appropriate.

Chronic toxicity

There are no individually tested chronic toxicity data available for (Z)-1,3-dichloropropene (Cis isomer) or (E)-1,3-dichloropropene (Trans isomer). There are no indications from the acute toxicity testing that the isomers differ in ecotoxicity, with all comparable species results being in the same order of magnitude. The mixture of isomers has been tested extensively at a chronic timescale. Therefore, both have been inherently tested within the mixture of isomers.

Concluding comments

Based on a read-across, category-based evaluation of structural and physical-chemical information and the toxicological and ecotoxicological properties of 1,3-dichloropropene (mixture of cis and trans isomers), (Z)-1,3-dichloropropene (cis isomer) and (E)-1,3-dichloropropene) grouping these substances in one group for the purposes of establishing a harmonized classification according to Regulation (EC) No. 1272/2008 (the CLP Regulation) is considered to be justified.

14.2 Annex II (Confidential Annex): Full reference list including study authors

See separate document.