ECHA
EUROPEAN CHEMICALS AGENCY

Committee for Risk Assessment
RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

Dimethyl disulphide

EC Number: 210-871-0
CAS Number: 624-92-0

CLH-O-0000001412-86-218/F

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

Adopted
8 June 2018

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CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Dimethyl disulphide

EC Number:	**210-871-0**
CAS Number:	**624-92-0**
Index Number:	**/**

Contact details for dossier submitter: ARKEMA France

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CONTENTS

1 IDENTITY OF THE SUBSTANCE 1
1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE 1
1.2 COMPOSITION OF THE SUBSTANCE 1
2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING 3
2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA 3
3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING 5
4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL 5
5 IDENTIFIED USES 6
6 DATA SOURCES 7
7 PHYSICOCHEMICAL PROPERTIES 9
8 EVALUATION OF PHYSICAL HAZARDS 10
8.1 FLAMMABLE LIQUIDS 10
8.1.1 Short summary and overall relevance of the provided information on flammable liquids 10
8.1.2 Comparison with the CLP criteria 10
8.1.3 Conclusion on classification and labelling for flammable liquids 10
9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 10
9.1 SHORT SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED TOXICOKINETIC INFORMATION ON THE PROPOSED CLASSIFICATION(S) 11
10 EVALUATION OF HEALTH HAZARDS 14
10.1 ACUTE TOXICITY - ORAL ROUTE 14
10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity 16
10.1.2 Comparison with the CLP criteria 16
10.1.3 Conclusion on classification and labelling for acute oral toxicity 16
10.2 ACUTE TOXICITY - DERMAL ROUTE 17
10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity 18
10.2.2 Comparison with the CLP criteria 18
10.2.3 Conclusion on classification and labelling for acute dermal toxicity 18
10.3 ACUTE TOXICITY - INHALATION ROUTE 18
10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity 18
10.3.2 Comparison with the CLP criteria 19
10.3.3 Conclusion on classification and labelling for acute inhalation toxicity 19
10.4 SKIN CORROSION/IRRITATION 22
10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation 23
10.4.2 Comparison with the CLP criteria 23
10.4.3 Conclusion on classification and labelling for skin corrosion/irritation 23
10.5 SERIOUS EYE DAMAGE/EYE IRRITATION 24
10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation 26
10.5.2 Comparison with the CLP criteria 26
10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation 26
10.6 RESPIRATORY SENSITISATION 28
10.7 SKIN SENSITISATION 28
10.7.1 Short summary and overall relevance of the provided information on skin sensitisation 29
10.7.2 Comparison with the CLP criteria 32
10.7.3 Conclusion on classification and labelling for skin sensitisation 32

10.8 GERM CELL MUTAGENICITY ... 36
10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity ... 40
10.8.2 Comparison with the CLP criteria ... 41
10.8.3 Conclusion on classification and labelling for germ cell mutagenicity ... 42
10.9 CARCINOGENICITY ... 44
10.10 REPRODUCTIVE TOXICITY ... 44
10.10.1 Adverse effects on sexual function and fertility ... 44
10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility ... 46
10.10.3 Comparison with the CLP criteria ... 47
10.10.4 Adverse effects on development ... 47
10.10.5 Short summary and overall relevance of the provided information on adverse effects on development 48
10.10.6 Comparison with the CLP criteria ... 49
10.10.7 Adverse effects on or via lactation ... 49
10.10.8 Conclusion on classification and labelling for reproductive toxicity ... 49
10.11 SPECIFIC TARGET ORGAN TOXICITY-SINGLE EXPOSURE ... 75
10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure ... 75
10.11.2 Comparison with the CLP criteria ... 77
10.11.3 Conclusion on classification and labelling for STOT SE ... 77
10.12 SPECIFIC TARGET ORGAN TOXICITY-REPEATED EXPOSURE ... 82
10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure ... 84
10.12.2 Comparison with the CLP criteria ... 85
10.12.3 Conclusion on classification and labelling for STOT RE ... 85
10.13 ASPIRATION HAZARD ... 91

11 EVALUATION OF ENVIRONMENTAL HAZARDS ... 91

11.1 RAPID DEGRADABILITY OF ORGANIC SUBSTANCES ... 91
11.1.1 Ready biodegradability ... 93
11.1.2 Degradation simulation testing in sediment (aerobic) ... 93
11.1.3 Degradation simulation testing in soil (aerobic) ... 93
11.1.4 Hydrolysis ... 93
11.1.5 Conclusion on rapid degradability according to CLP criteria ... 94
11.2 BIOACCUMULATION ... 94
11.2.1 Measured partition coefficient and bioaccumulation test data ... 95
11.2.2 Conclusion on bioaccumulation according to CLP criteria ... 95
11.3 ACUTE AQUATIC HAZARD ... 95
11.3.1 Acute (short-term) toxicity to fish ... 98
11.3.2 Acute (short-term) toxicity to aquatic invertebrates ... 99
11.3.3 Acute (short-term) toxicity to algae or other aquatic plants ... 99
11.4 LONG-TERM AQUATIC HAZARD ... 100
11.4.1 Chronic toxicity to fish ... 101
11.4.2 Chronic toxicity to aquatic invertebrates ... 102
11.4.3 Chronic toxicity to algae or other aquatic plants ... 103
11.5 COMPARISON WITH THE CLP CRITERIA ... 104
11.5.1 Acute aquatic hazard ... 104
11.5.2 Long-term aquatic hazard (including bioaccumulation potential and degradation) ... 104
11.6 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS ... 104

12 REFERENCES ... 109

12.1 REFERENCES QUOTED IN THE REGISTRATION DOSSIER AND THE CHL REPORT ... 109
12.2 REFERENCES QUOTED IN THE REGISTRATION DOSSIER AND NOT QUOTED IN THE CHL REPORT ... 115

13 ANNEXE I ... 119

1 PHYSICAL HAZARDS ... 5

1.1 FLAMMABLE LIQUID ... 5

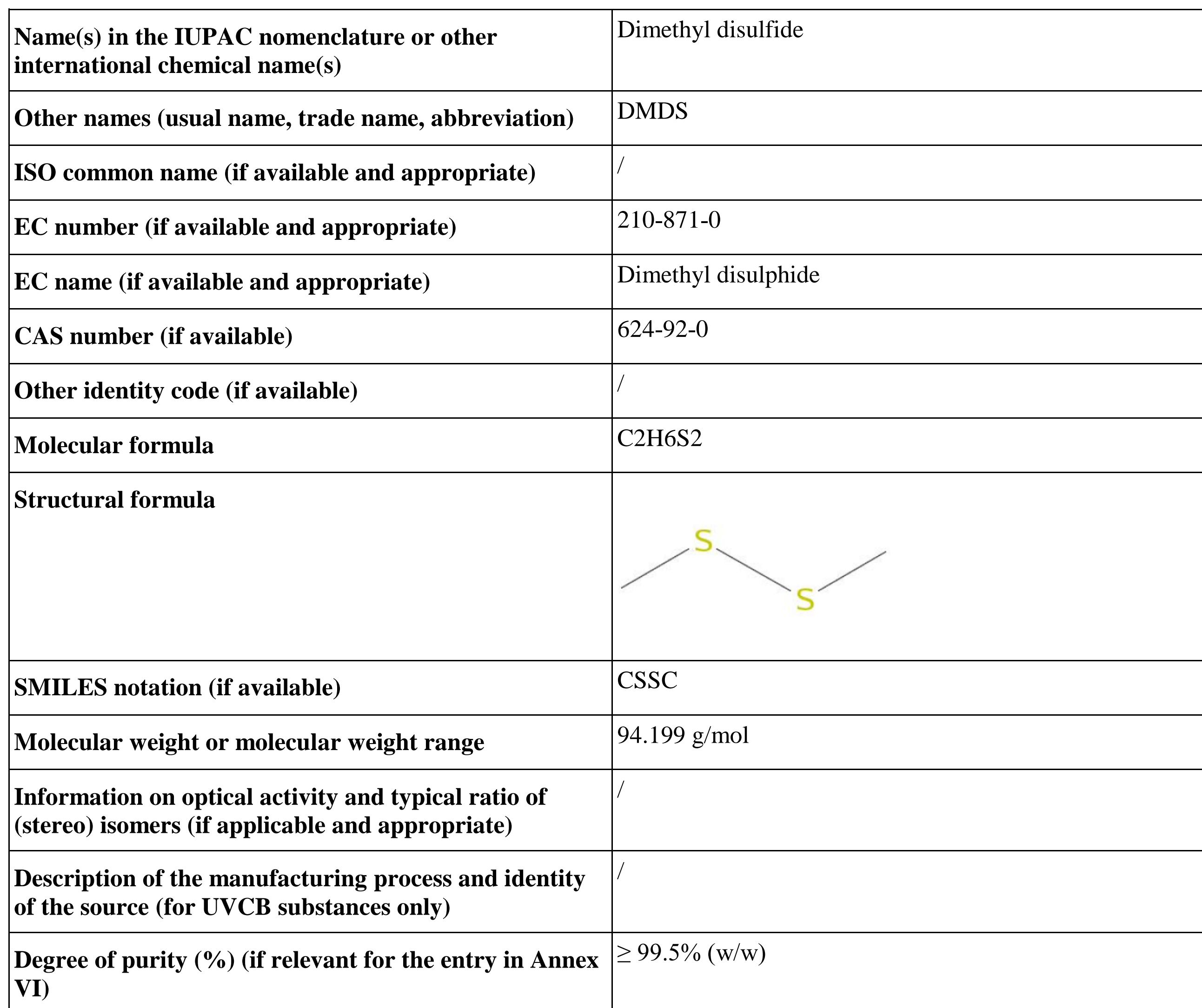
2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) ... 6

3 HEALTH HAZARDS 19
3.1 ACUTE TOXICITY - ORAL ROUTE 19
3.2 ACUTE TOXICITY - DERMAL ROUTE 32
3.3 ACUTE TOXICITY - INHALATION ROUTE 43
3.4 ACUTE TOXICITY – OTHER ROUTES 69
3.5 SKIN CORROSION/IRRITATION 69
3.6 SERIOUS EYE DAMAGE/EYE IRRITATION 76
3.7 SKIN SENSITISATION 84
3.8 REPEATED DOSE TOXICITY – INHALATION ROUTE 95
3.9 REPEATED DOSE TOXICITY – DERMAL ROUTE 117
3.10 GERM CELL MUTAGENICITY 121
3.10.1 In vitro data 121
3.10.2 In vivo 140
3.11 REPRODUCTIVE TOXICITY 153
3.11.1 Toxicity to reproduction 153
3.11.2 Development toxicity – Teratogenicity 180
3.11.3 Other data 197
3.12 NEUROTOXICITY 199
3.13 IMMUNOTOXICITY 209
4 ENVIRONMENTAL HAZARDS 216
4.1 STABILITY 216
4.1.1 Hydrolysis 216
4.2 BIODEGRADATION 219
4.2.1 Biodegradation in water: screening tests 219
4.2.2 Degradation simulation testing in sediment 223
4.3 BIOACCUMULATION 227
4.3.1 Bioaccumulation: aquatic / sediment 227
4.4 ACUTE TOXICITY 228
4.4.1 Short-term toxicity to fish 228
4.4.2 Short-term toxicity to aquatic invertebrates 236
4.4.3 Algal growth inhibition tests 249
4.5 CHRONIC TOXICITY 263
4.5.1 Fish early-life stage (FELS) toxicity test 263
4.5.2 Chronic toxicity to aquatic invertebrates 269

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)	Dimethyl disulfide
Other names (usual name, trade name, abbreviation)	DMDS
ISO common name (if available and appropriate)	/
EC number (if available and appropriate)	210-871-0
EC name (if available and appropriate)	Dimethyl disulphide
CAS number (if available)	624-92-0
Other identity code (if available)	/
Molecular formula	C2H6S2
Structural formula	
SMILES notation (if available)	CSSC
Molecular weight or molecular weight range	94.199 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	/
Description of the manufacturing process and identity of the source (for UVCB substances only)	/
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 99.5% (w/w)

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 3.1 (CLP)	Current self-classification and labelling (CLP)
Dimethyl disulphide **EC no.: 210-871-0**	≥ 99.5% (w/w)	Not applicable	Flam. Liquid 2, H225 Acute Tox. 4; H302 Acute Tox. 3; H331 Eye Irrit. 2; H319 Skin Sens. 1; H317 STOT SE 3; H335 Aquatic Acute 1; H400

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
			(M=1) Aquatic Chronic 1; H410 (M=10)

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 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
Methanethiol **EC no.: 200-822-1**	≤ 0.01% (w/w)	Press. Gas; H280 Flam. Gas 1; H220 Acute Tox. 3*; H331 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Press. Gas; H280 Flam. Gas 1; H220 Acute Tox. 3*; H331 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	No contribution at this concentration
Confidential impurity 1	≤ 0.15% (w/w)			No
Water **EC no.: 231-791-2**	≤ 0.06% (w/w)			No
Unknown impurity	≤ 0.28% (w/w)			No

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 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
No additives					

Table 5: Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**	**The study(ies) in which the test substance is used**
Dimethyl disulphide **EC no.: 210-871-0**	98%	Methanethiol (EC: 200-822-1): 1%		Shapiro, R (1986)
Dimethyl disulphide **EC no.: 210-871-0**	98%	Methanethiol (EC: 200-822-1): 1%		Haynes, G (1988)
Dimethyl disulphide **EC no.: 210-871-0**	99.31%	Methanethiol (EC: 200-822-1): 0.46%		Lombard, A (1986)
Dimethyl disulphide **EC no.: 210-871-0**	98.98%	Methanethiol (EC: 200-822-1): 0.26%		Guillot, JP (1985)
Dimethyl disulphide **EC no.: 210-871-0**	98%	Methanethiol (EC: 200-822-1): 1%		Shapiro, R (1985b)

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	No current Annex VI entry										
Dossier submitters proposal		Dimethyl disulphide	210-871-0	624-92-0	Flam. Liquid 2 Acute Tox. 4 Acute Tox. 3 Eye Irrit. 2 Skin Sens. 1 STOT SE 3 Aquatic Acute 1 Aquatic Chronic 1	H225 H302 H331 H319 H317 H335 H400 H410	GHS02 GHS06 GHS09 Dgr	H225 H302 H331 H319 H317 H335 H410	/	M acute = 1 M chronic = 10	
Resulting Annex VI entry if agreed by RAC and COM		Dimethyl disulphide	210-871-0	624-92-0	Flam. Liquid 2 Acute Tox. 4 Acute Tox. 3 Eye Irrit. 2 Skin Sens. 1 STOT SE 3 Aquatic Acute 1 Aquatic Chronic 1	H225 H302 H331 H319 H317 H335 H400 H410	GHS02 GHS06 GHS09 Dgr	H225 H302 H331 H319 H317 H335 H410	/	M acute = 1 M chronic = 10	

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	No
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 conclusive but not sufficient for classification	No
Pyrophoric liquids	Data conclusive but not sufficient for classification	No
Pyrophoric solids	Hazard class not applicable	No
Self-heating substances	Data conclusive but not sufficient for classification	No
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	No
Oxidising liquids	Data conclusive but not sufficient for classification	No
Oxidising solids	Hazard class not applicable	No
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data conclusive but not sufficient for classification	No
Acute toxicity via oral route	Harmonised classification proposed	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Harmonised classification proposed	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Harmonised classification proposed	Yes
Respiratory sensitisation	No data are available for that specific hazard class	No
Skin sensitisation	Harmonised classification proposed	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	No data are available for that specific hazard class	No
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Harmonised classification proposed	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Data conclusive but not sufficient for classification	Yes

Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Data lacking	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

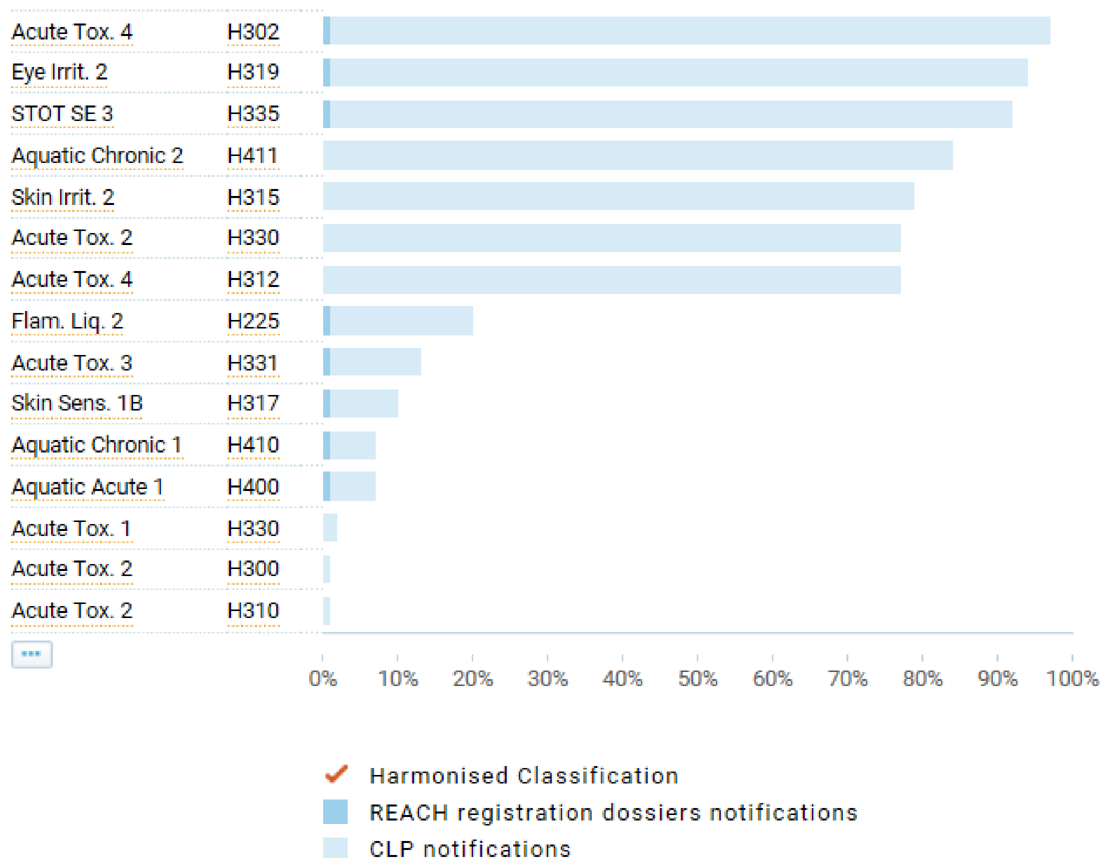
Not applicable.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Dimethyl disulphide (DMDS) is an industrial chemical manufactured, imported and used in Europe in large quantities as intermediate for chemical synthesis, processing aid in refineries and petrochemical sites. Available information indicates that DMDS fulfils hazard classes other than those listed in Article 36(1) of Regulation (EC) N°1272/2008. No entry in Annex VI in the same Regulation exist for this substance.

Dimethyl disulphide was first registered under Regulation EC 1907/2006 (REACH) in 2010. Arkema France, as major European produced and largest data holder, acted as Lead Registrant and prepared the registration dossier and a proposal for self –classification. This was approved by co-registrants and circulated to all SIEF members on the 9th June 2010. Arkema France also communicated to SIEF members on the 13th August 2012 and 5th March 2013 evolution in the self-classification of DMDS justified by new data.

Despite these efforts to facilitate a common self-classification for DMDS in Europe, the public dissemination web site of ECHA shows that there are still differences in the self-classification supported by the joint registration by Arkema France and that supported by another registrant not part of the Joint Registration. There are two distincts entries for the CLP inventory (one from Arkema France and co-registrants and the other from 2 other entities). Differences in classification can also be seen in CLP notifications (more than 950 entries).



In order to resolve these differences which may confuse the understanding about the hazards by the users and the free flow of goods in Europe, in light of the recent efforts of the German Member State in assessing the hazardous properties of DMDS summarized in Arkema France's joint registration dossier, Arkema France and co-registrants consider that the need for proposing a harmonised classification and labelling according to Article 36(3) is justified at community level.

Arkema France as Lead Registrant for DMDS and with the support of co-registrants, therefore respectfully submits the proposal of harmonised classification and labelling in application of Articles 37(2) and 37(3) of Regulation (EC) N° 1272/2008.

- DMDS is proposed in parallel as an active substance under Regulation (EC) 1107/2009 : as it was not yet approved as such, the conditions of Article 36/2) are not considered to be met in 2016.

5 IDENTIFIED USES

Dimethyl disulphide (DMDS) is manufactured and processed almost entirely within industrial closed systems. Its major uses are:

- Use as hydrotreating catalyst activator in oil refining: optimal maintenance of production units. DMDS is used in oil refineries as a sulfiding/presulfiding agent to activate the catalyst of hydrotreating units.

- Use in petrochemical industry to reduce the number of decoking operations. Operators in the petrochemicals industry must protect their steamcracking coils against the formation of coke and carbon monoxide.

- Use as intermediate in synthesis of fine chemicals

6 DATA SOURCES

Information on Dimethyl disulfide (DMDS) was collected from different sources.The last updated compilation was done on 2009.11.02. After this date, information has been regularly updated.

1) External Databases interrogated for the Literature Search:

a) (Eco)toxicology Literature Search (2001.10.01)
The search was performed by CAS number (624-92-0).
Databases: Toxline, Toxlit, Chemlist, SANSS, TSCATS, AQUIRE, BIOLOG, CESAR, DATALOG, ENVIROFATE, ISHOW, NIOSHTIC SUBSET, PHYTOTOX

b) This substance is followed up by SDI (Selective Diffusion of Information) from 1997 to today.
A specific profile for Mercaptans has been created, including dimethyl disulfide.

Search strategy : request by CAS number / weekly SDI

1997-2002 : SDI profile created from TOXLINE/TOXLIT, two National Library of Medicine's bibliographic databases containing records with international coverage in the biochemical, pharmacological, physiological and toxicological areas.

2002 – today: SDI profile continued on TOXCENTER, the Scientific and Technical Information Network's bibliographic database, produced by Chemical Abstracts Service.
TOXCENTER has replaced TOXLINE and TOXLIT when STN decided to remove the both databases from its Network.

c) Physico-Chemical Literature Search (2009.11.02)

Each search was performed by CAS number (624-92-0) or by synonyms (Dimethyl disulfide, Methyl disulfide, DMDS)
- eChemPortal (covering CESAR, CHRIP, EnviChem, ESIS, HPVIS, HSNO CCID, INCHEM, JECDB, NICNAS, PEC, OECD HPV, SIDS IUCLID, SIDS UNEP, US EPA IRIS, and US EPA SRS)
Location : http://webnet3.oecd.org/echemportal/Home.aspx
- BEILSTEIN, CHEMSAFE, CSNB, MRCK, REGISTRY

d) Other search engines:
The search was performed by CAS number.
- Expert Publishing - Location: http:// www.expub.com
- TOXNET (covering ChemIDplus, HSDB, TOXLINE, CCRIS, DART, GENETOX, IRIS, ITER, LactMed, TRI, Haz-map, Household Products and TOXMAP) - Location: http://toxnet.nlm.nih.gov/

2) TOCs alerts
We receive the last Tables of Contents of the main journals in (eco)toxicology, as soon as publication on the editor's website. A systematic selection is done for substances, including DMDS.

3) Other potential sources on Internet

Each search was performed by CAS number or by synonyms: Dimethyl disulfide, Methyl disulfide, DMDS
- ATSDR (Agency for Toxic Substances and Disease Registry) - Location:
http://www.atsdr.cdc.gov/toxpro2.html#d
Document type: Toxicological Profiles
-ECB (European Chemicals Bureau)
Location: http://ecb.jrc.ec.europa.eu/esis/index.php?PGM=dat
Document types: IUCLID, Risk assessments
- ECETOC (European Centre for Ecotoxicoloy and Toxicology of Chemicals)
Location : http://www.ecetoc.org/publications
Document types : JACC Reports, Monographs, Special Reports or Technical Reports
- EPA (Environmental Protection Agency)
Location: http://www.epa.gov/chemrtk/pubs/summaries/viewsrch.htm
Document types : Test plans, Robust summaries
+ Database IRIS (Integrated Risk Information System) : http://www.epa.gov/ncea/iris/search_keyword.htm
- IARC (International Agency for Research on Cancer)
Location: http://monographs.iarc.fr/ENG/Monographs/allmonos90.php
Document type: Monographs
- INERIS (Institut National de l'EnviRonnement industriel et des rISques)
Location : http://www.ineris.fr/index.php?module=cms&action=getContent&id_heading_object=4
Document types: Fiches de données toxicologiques et environnementales des substances chimiques, Fiches & rapports de seuils de toxicité aiguë (French)
- INRS (Institut National de Recherche et de Sécurité)
Document type : Fiches toxicologiques (French)
- IPCS (The International Programme on Chemical Safety)
Location: http://www.who.int/ipcs/en/
Document types: CICADS Concise International Chemical Assessment Documents, EHC Environmental Health Criteria Monographs, HSG Health and Safety Guides, ICSC International Chemical Safety Cards
- JETOC (Japan Chemical Industry Ecology-Toxicology & Information Center)
Location : http://www.jetoc.or.jp/
Document type: Japan chemical industry ecology-toxicology & information center reports
- NIHS Japan (National Institute of Health Sciences)
Location: http://www.nihs.go.jp/english/usefulinfo/chemicals.html
Document type: Toxicity Testing Reports of Environmental Chemicals
- NIOSH (National Institute for Occupational Safety and Health)
Location : http://www.cdc.gov/niosh/docs/81-123/
Document types : Occupational Health Guidelines for Chemical Hazards, Criteria Documents
- NTP (National Toxicology Program)
Location : http://ntp-server.niehs.nih.gov/?objectid=7DA86165-BDB5-82F8-F7E4FB36737253D5
Document type : Reports
- OECD (Organisation for Economic Co-operation and Development)
Location: http://www.oecd.org/document/63/0,3343,en_2649_34379_1897983_1_1_1_1,00.html
Document types: SIDS draft final reports
- RAIS (Risk Assessment Information Systems)
Location : http://rais.ornl.gov/tox/rap_toxp.shtml
Document type : Toxicity profiles
- UNEP (United Nations Environment Programme)
Location : http://www.chem.unep.ch/irptc/sids/OECDSIDS/sidspub.html
Document type : SIDS publications

4) Textbooks and Monographs

- ACGIH : Threshold Limit Value (TLV®) occupational exposure guidelines, Biological Exposure Indices (BEIs®)
- AIHA: HGS Hygienic Guide Series, ERPG Emergency Response Planing Guidelines, WEELG Workplace Environmental Exposure Level Guides
- BG Chemie (BerufsGenossenschaft der chemischen Industrie): Toxicological evaluations
- BUA (GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance – Beratergremium für Umwelttrelevante Altstoffe): Reports
- DFG (Deutsche ForschungsGemeinschaft): Occupational toxicants, Critical data evaluation for MAK values and classification of carcinogens
- Main monographs / handbooks (Merck, Patty, Pluyette, Rippen, Sax, Verschuren, …)

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	Liquid, light yellow	Van Nostrand Reinhold (1984)	
Melting/freezing point	-84.7°C at 101.3 kPa	Diepenhorst (2005)	
Boiling point	109.2°C at 101.3 kPa	Diepenhorst (2005)	
Relative density	1.062 at 20°C	Diepenhorst (2006)	
Vapour pressure	30.0 hPa at 20°C 38.6 hPa at 25°C	Diepenhorst (2005)	
Surface tension	72.1 mN/m at 20°C and 1000 mg/l	Diepenhorst (2006)	
Water solubility	2.7 g/l at 20°C	Diepenhorst (2006)	
Partition coefficient n-octanol/water	1.91 at 20°C	Diepenhorst (2006)	
Flash point	15°C at 101.3 kPa	Mak (2005)	
Flammability	Highly flammable liquid		Based on flash point and boiling point
Explosive properties	Not explosive		Due to chemical structure (no chemical groups associated with explosive properties)
Self-ignition temperature	304°C at 101.3 kPa	Mak (2005)	
Oxidising properties	Not oxidising		Due to chemical structure (the substance is incapable of exothermic reaction with combustible materials)
Granulometry	Not applicable		
Stability in organic solvents and identity of relevant degradation products	Not relevant		
Dissociation constant	No dissociation observed	Diepenhorst (2006)	
Viscosity	0.59 mm2/ at 20°C 0.49 mm2/s at 40°C	Gancet (2010)	

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Flammable liquids

Table 9: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
Closed cup EU Method A.9 (Flash-Point)	Flash point: 15 °C at 101.282 kPa	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Mak WA (2005)
Determination of flash point closed cup	Flash point: 24 °C	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): dimethyl disulphide**	Dean JA (1987)

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

According to an experimental key study, the flash point of dimethyl disulphide was measured at 15°C at 101.282 kPa (according to guideline EU Method A.9).

8.1.2 Comparison with the CLP criteria

For liquids, the flammability is derived from the flash point and the boiling point of the substance. As dimethyl disulphide has a flash point below 23°C and a boiling point above 35°C (measured at 109.2°C), is considered as highly flammable (category 2).

8.1.3 Conclusion on classification and labelling for flammable liquids

In accordance with Regulation (EC) No 1272/2008, dimethyl disulfide shall be classified as Flammable Liquid 2 (Hazard statement: H225; Highly flammable liquid and vapour)

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 10: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
in vitro study rat (Sprague-Dawley) male/female in vitro	Metabolites identified: yes Details on metabolites: Methyl mercaptan and dimethyl sulphide Evaluation of results: bioaccumulation	1 (reliable without restriction) key study experimental result	Kilford P (2012)

Method	Results	Remarks	Reference
To identify the number and proportions of its metabolites, dimethyl disulphide was incubated with fresh rat hepatocytes. Samples were removed after 0, 1, 2 and 4 hours and quenched in liquid nitrogen. All samples were analysed by GC-MS.	potential cannot be judged based on study results	**Test material (EC name): dimethyl disulphide** Form: liquid	

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

No Good Laboratory Practice- (GLP-) and test guideline-compliant oral administration in vivo metabolism studies are available for dimethyl disulphide (DMDS). Nevertheless there is a wealth of data in the public domain on the natural occurrence of DMDS in the diet, the use of DMDS as a flavouring agent and the absorption, excretion, distribution and metabolism of simple disulphides following ingestion. Some supporting information is also available on thein vitrometabolism of DMDS in rat hepatocytes (Kilford, 2012, summarised at the end of this section) and on the disposition and metabolism of an analogous simple disulphide, dipropyl disulphide, in the rat after oral administration. A study on pulmonary excretion of DMDS in mice when administered by intraperitoneal injection is also available.

DMDS has been investigated by the World Health Organization (WHO) and European Food Safety Authority (EFSA) in respect to its use as a food flavouring agent. An evaluation of absorption, metabolism and elimination of DMDS was described in WHO Food Additive Series No. 59 (Williams GM & Bend J (2000)). DMDS was assigned to the group of substances ‘simple disulphides’, which are of low relative molecular mass and are sufficiently lipophilic to be absorbed from the intestine. As metabolism would usually result in increased polarity and a greater likelihood of excretion, these substances would not be expected to accumulate in the body. Disulphides would be able to form disulphide bonds with endogenous thiols. Disulphides formed with cysteine could be excreted in the urine as cysteine disulphide, whereas formation of disulphides with endogenous macromolecules would be eliminated more slowly.

The reduction of simple disulphides is believed to be extensive, and the reaction may be catalysed enzymatically by thioltransferases and chemically by exchange with glutathione, thioredoxin, cysteine and other endogenous thiols. Reduction of non-cyclic disulphides, such as dimethyl disulphide, would result in the formation of thiols of low relative molecular mass, which would then be metabolised by the various pathways described below for simple thiols.

Simple thiols can be metabolised via several pathways. Simple aliphatic thiols undergoS-methylation in mammals to produce the corresponding methyl thioether or sulphide. S-Methylation is catalysed by thiopurineS-methyltransferase in the cytosol and thiolS-methyltransferase in microsomes; both reactions requireS-adenosyl-L-methionine as a methyl group donor. ThiopurineS-methyltransferase is present in human liver, kidney and erythrocytes, and its preferred substrates include aromatic and heterocyclic thiols. S-Methylation of aliphatic thiols is catalysed by microsomal thiol S-methyltransferase, and the resulting methyl thioether (sulphide) metabolite undergoesS-oxidation to give the corresponding methyl sulphoxide and methyl sulphone analogues, which are excreted in the urine.

Thiols may react with glutathione and other endogenous thiol substances to form mixed disulphides. Both microsomal and cytosolic thioltransferases have been reported to catalyse the formation of mixed

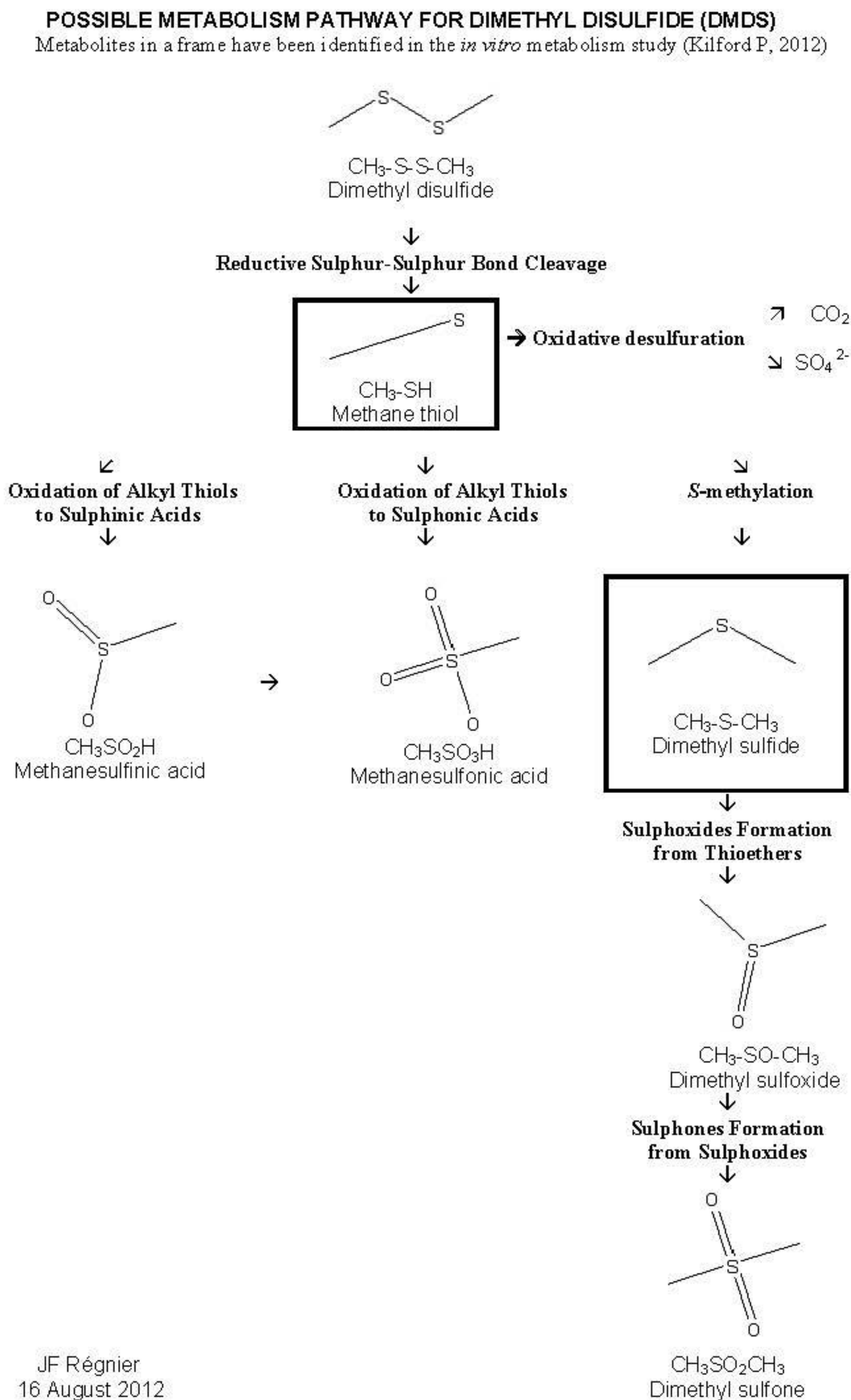
disulphides. The resulting mixed disulphides can undergo reduction back to thiols, oxidative desulphuration or oxidation to the corresponding sulphonic acid via the intermediate thiosulphinate and sulphinic acid. The principal form in the circulation would probably be a mixed disulphide formed with albumin.

Thiols may be oxidised to form sulphenic acids (RSOH), which are unstable and readily undergo further oxidation to sulphinic (RSO2H) and sulphonic (RSO3H) acids or combine with nucleophiles. The sulphonic acid group is highly polar and renders molecules very soluble in water. In general, sulphonic acids are not extensively metabolised.

Alkyl thiols of low relative molecular mass undergo oxidative desulphuration in vivo to yield carbon dioxide and sulphate. This reaction has been shown to occur, for example, with methyl mercaptan. Whereas the carbon atoms from thiols may be used in the biosynthesis of amino acids, the sulphur atoms are not used significantly in the synthesis of sulphur-containing amino acids.

A possible metabolism pathway for dimethyl disulphide is shown in the following Figure:

Figure: Possible metabolism pathway for DMDS



An in vitro metabolism study in rat hepatocytes has been performed and has been summarised at the end of this section (Kilford, 2012). The objective of this study was to identify the metabolites that were generated following incubation of DMDS with fresh rat hepatocytes. The study demonstrated rapid metabolism with the formation of methane thiol (also known as methyl mercaptan) and dimethyl sulphide at lower levels. This correlates with the reduced sulphur-sulphur bond cleavage and theS-methylation steps presented in the metabolism pathway shown above and confirms the role of the liver in the metabolism of DMDS.

The metabolism of another simple disulphide and member of WHO TRS 896 Subgroup vii – simple disulphides, structural class I (WHO TRS 896), dipropyl disulphide (DPDS) was investigated in vivo in the rat after a single oral administration (Germain et al., 2008). DPDS was detected in the stomach where it was transformed into propyl mercaptan, whereas the liver contained only traces of DPDS and none at all in blood and intestines. The metabolites methylpropyl sulphide, methylpropyl sulphoxide (MPSO), and methylpropyl sulphone (MPSO2) were sequentially formed in the liver. The route of elimination from the liver seemed to be mainly via the blood. The bile also participated in the excretory process, but only for MPSO2. The pharmacokinetic parameters were determined for all of the above compounds. Whereas the bioavailability of DPDS, measured by the area under the curve (AUC) of each compound was very low (0.008 h mM), the AUCs were higher for the S-oxidized metabolites MPSO and MPSO2, i. e. 9.64 and 24.15 h mM, respectively. The half-lives for DPDS and its metabolites varied between 2.0 and 8.25 h, except for MPSO2, which had a half-life of 29.6 h. MPSO2was the most abundant and persistent of these metabolites. This study confirmed the literature information that the liver plays a major role in thein vivometabolism of DPDS and all metabolites except propyl mercaptan first appeared in the liver. The slow elimination of MPSO2(half-life = 29.57h) and the level of this molecule in the liver suggests a role for this organ in the storage of sulphur compounds.

In mice that were injected intraperitoneally with 35 to 40 mg DMDS/kg (Susman et al, 1978), three volatile sulfur compounds were detected in breath samples: DMDS itself (parent compound), dimethyl sulphide (metabolite) and methyl mercaptan (metabolite). The excretion of the parent compound, DMDS, reached a peak between 3 and 6 minutes following injection, and the total amount excreted was approximately 6% of the administered dose. The amounts of the two metabolites accounted for approximately 0.5% each of the administered dose. The study concluded that pulmonary excretion appeared to be quantitatively insignificant as a means for the elimination of sulphide by mice.

DMDS occurs naturally in a large number of food types and may also be ingested through its use as a food flavouring agent. In WHO Food Additive Series 59, consideration was made of combined intakes from use of flavouring agents and natural occurrence. It was concluded that under the current conditions of use as flavouring agents, the combined intakes of these substances would not saturate the metabolic pathways and combined intakes would not raise safety concerns. The estimated current intake in Europe from use as food flavouring agent is 11 µg/person/day (WHO TRS 896) whilst the threshold level of concern for structural class I is 1800 µg/person/day. Safe use of DMDS as a flavouring agent has been accepted by EFSA (EFSA Journal 2011; 9(12):2459) in the absence of DMDS-specific animal metabolism data.

DMDS can be absorbed through the skin, however, no dermal penetration data are available.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 11: Summary table of animal studies on acute oral toxicity

Method	Results	Remarks	Reference
rat (Sprague-Dawley) female oral: gavage OECD Guideline 423 (Acute Oral	LD0: >= 300 mg/kg bw (female) (no mortality, clinical signs included hypoactivity, hypersalivation, piloerection and dyspnea)	1 (reliable without restriction) key study	Pelcot C (2010)

Method	Results	Remarks	Reference
toxicity - Acute Toxic Class Method) EU Method B.1 tris (Acute Oral Toxicity - Acute Toxic Class Method)		experimental result **Test material (EC name): dimethyl disulphide**	
rat (Sprague-Dawley) male/female oral: gavage EU Method B.1 (Acute Toxicity (Oral))	LD50: 385 (290 – 500) mg/kg bw (male/female) (Post-exposure clinical signs: sedation, hypotonia, dyspnea, piloerection and coma)	1 (reliable without restriction) supporting study experimental result **Test material (EC name): dimethyl disulphide**	Lombard A (1986)
rat [common species] (Wistar [rat]) female oral: gavage according to OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method)	LD50: <501 mg/kg bw (female)	1 (reliable without restriction) other information experimental study **Test material (EC name): dimethyl disulphide**	Gilotti AC 2006
rat [common species] (Wistar [rat]) male/female oral: gavage according to OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method)	LD100: 500 mg/kg bw (female) (No animals survived. Predeath signs: wetness of the nose/mouth area, flaccid muscle tone, prostration, negative righting reflex and coma. At necropsy: abnormalities of the lungs, thymus and liver, as well as wetness of the nose/mouth area.) LD0: >56 mg/kg bw (male/female) (No mortality. Instances of wetness of the nose/mouth area, lethargy, sagging eyelids, negative righting reflex, ataxia and wetness of the anogenital area in the males not in the females)	1 (reliable without restriction) other information experimental study **Test material (EC name): dimethyl disulphide**	Gilotti AC 2007
rat [common species] (Wistar [rat]) male/female oral: gavage according to EPA 40 CFR 163.81-1	LD50: 190 mg/kg bw (male/female) (Post-exposure clinical signs: sedation, hypotonia, dyspnea, piloerection and coma)	4 (not assignable) disregarded due to major methodological deficiencies experimental study **Test material (EC name): dimethyl**	Shapiro R 1985a

Method	Results	Remarks	Reference
		disulphide	
rat [common species] (Wistar [rat]) male/female oral: gavage according to EPA 40 CFR 163.81-1	LD100: <5000 mg/kg bw (male/female) (Thirty minutes after the administration, all the animals were dead.)	4 (not assignable) disregarded due to major methodological deficiencies experimental study **Test material (EC name): dimethyl disulphide**	Shapiro R 1985b

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

In a key study, the acute oral toxicity of dimethyl disulphide (purity 99.88%) was evaluated in rats according to OECD No. 423 (17th December 2001) and Council Regulation (EC) No. 440/2008, B.1tris(30 May 2008) guidelines (Pelcot, 2010). The study was conducted in compliance with the principles of Good Laboratory Practice Regulations. Dimethyl disulphide was administered in corn oil by oral route (gavage), under a volume of 10 mL/kg to 6 fasted female Sprague-Dawley rats. Clinical signs, mortality and body weight gain were checked for a period of up to 14 days following the single administration. All animals were subjected to necropsy. No deaths occurred. Hypoactivity, dyspnea and hypersalivation were noted in all animals 10 minutes after treatment. Hypoactivity persisted in 4/6 animals until 4 hours after treatment. In addition, piloerection and dyspnea were observed in one animal 3 hours after treatment and in another one 4 hours after treatment. When compared to historical control animals, a lower body weight gain was noted in 3/6 animals between day 1 and day 8, persisting in one of them between day 8 and day 15. The body weight gain of the other animals was not affected by treatment with the test item. The oral LD0 of dimethyl disulphide was higher than 300 mg/kg in rats.

In a supporting study, the acute oral toxicity of dimethyl disulphide (DMDS, purity 99.31%) was evaluated in male and female Sprague-Dawley rats according to EU Method B.1 (Lombard, 1986). Animals were treated with dose levels of 100, 170, 290, 350, 500 and 5300 mg/kg (5 ml/kg) and then observed for 14 days for mortality, clinical signs and effect on body weight. No mortality was observed at the dose levels of 100, 170 and 350 mg/kg. At 290 mg/kg the mortality was 30% and 100% at 500 and 5300 mg/kg. Sedation, hypotonia, dyspnea, piloerection and coma, appeared just after the administration and disappeared after 24 hours. No effect was noted on the body weight gain of the surviving rats. Haemorragic stomachs was observed at the macroscopic examination of the rats dead on the first day (290 and 500 mg/kg). Under these experimental conditions, the oral LD50 of DMDS is between 290 and 500 mg/kg in female and male rats and an approximate LD50 of 385 mg/kg was calculated according to the standard probit method.

10.1.2 Comparison with the CLP criteria

The oral LD50 of DMDS in rats was higher than 300 mg/kg or equal to 385 mg/kg.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

In accordance with Regulation (EC) No 1272/2008, as the oral LD_{50} is between 300 and 2000 mg/kg, DMDS shall be classified as Acute Tox. 4 (Hazard statement: H302; Harmful if swallowed)

10.2 Acute toxicity - dermal route

Table 12: Summary table of animal studies on acute dermal toxicity

Method	Results	Remarks	Reference
rat (Wistar) male/female Coverage: semiocclusive Vehicle: unchanged (no vehicle) EPA OPPTS 870.1200 (Acute Dermal Toxicity)	LD0: > 5000 mg/kg bw (male/female) (No mortality. Instances of wetness and soiling of the anogenital area, chromorhinorrhea, sagging eyelids, emaciated appearance, few feces, lethargy, ataxia, wet red substance on anogenital area and nose/mouth area were noted during the study)	1 (reliable without restriction) weight of evidence experimental result **Test material (EC name): dimethyl disulphide**	Gilotti AC (2007)
rabbit (New Zealand White) male/female Coverage: occlusive Vehicle: unchanged (no vehicle) EPA OPPTS 870.1200 (Acute Dermal Toxicity)	LD0: >= 2000 mg/kg bw (male/female) (Post exposure clinical signs: heavy breathing, loss of righting reflex, spontaneous spasms, pupillary dilation and constriction, unwillingness to stand, lethargy, excessive salivation and flared nostrils.)	4 (not assignable) weight of evidence experimental result **Test material (EC name): dimethyl disulphide**	Shapiro R (1986)
rabbit (New Zealand White) male/female Coverage: occlusive Vehicle: unchanged (no vehicle) EPA 40 CFR 163.81-2	LD0: >= 2000 mg/kg bw (male/female) (Post-exposure clinical signs: none)	4 (not assignable) weight of evidence experimental result **Test material (EC name): dimethyl disulphide**	Shapiro R (1985c)
rabbit (New Zealand White) male/female Vehicle: none EU Method B.3 (Acute Toxicity (Dermal))	LD0: >= 2000 mg/kg bw (male/female) (Post-exposure clinical signs: apathy and prostration)	4 (not assignable) weight of evidence experimental result **Test material (EC name): dimethyl disulphide**	Sheppard DB (1985)
rabbit (New Zealand White) male/female Coverage: occlusive Vehicle: unchanged (no vehicle) EPA OPP 81-2 (Acute Dermal Toxicity)	LD0: >= 2000 mg/kg bw (male/female) (No mortality)	4 (not assignable) weight of evidence experimental result **Test material (EC name): dimethyl disulphide** Form: liquid	Haynes G (1988)

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

Four acute dermal toxicity studies were performed in rabbits with neat dimethyl disulphide (Shapiro, 1986 and 1985c; Sheppard, 1985; Haynes, 1988) following methods comparable to the OECD guideline #402, however, the information of on the analytical purity of the tested samples is missing. In these three studies, no mortality was observed at the limit dose level of 2000 mg/kg.

Additional information is provided by an acute dermal toxicity study in rats performed with a dimethyl disulphide formulation and according to OPPTS 870.1200 (August 1998) guideline and GLP. In this study, five male and female Wistar Albino rats were dosed dermally with dimethyl disulphide 94:5:1 (94% DMDS technical grade (purity 99.2%) with 5% emulsifier and 1% odorant) at 5000 mg/kg of body weight. The test article was kept in contact with the skin for 24 hours. Clinical signs, skin reactions, mortality and body weight gain were checked for a period of up to 14 days following the single administration. All animals were subjected to necropsy. All ten animals survived the 5000 mg/kg dermal application. Instances of wetness and soiling of the anogenital area, chromorhinorrhea, sagging eyelids, emaciatedappearance, few feces, lethargy, ataxia, wet red substance on anogenital and nose/mouth areas were noted during the study. Dermal effects ranged from absent to very slight on Day 1, absent to severe on Day 7 and absent to veryslight on Day 14. Two animals lost weight from Day 0 to Day 7, but gained weight overall by study termination. All other bodyweight changes are normal. Necropsy revealed abnormalities of the treated skin and thymus. Seven animals were normal at necropsy. The dermal LD0of dimethyl disulphide 94:5:1 is greater than 5000 mg/kg of body weight.

10.2.2 Comparison with the CLP criteria

Overall, on a weight of evidence approach, it can be concluded that the acute dermal LD_0 of dimethyl disulphide is higher than 2000 mg/kg in rats and rabbits.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

In accordance with Regulation (EC) No 1272/2008, as the dermal LD_{50} is ≥ 2000 mg/kg, no classification is warranted for DMDS.

10.3 Acute toxicity - inhalation route

Table 13: Summary table of animal studies on acute inhalation toxicity

Method	Results	Remarks	Reference
rat (Sprague-Dawley) male/female inhalation: vapour (whole body) EPA OPPTS 870.1300 (Acute inhalation toxicity)	LC50 (4 h): 1310 ppm (male/female) ((5.05 (4.49-5.66) mg/l) Clinical signs consisted of rales, decreased defecation and dried material on various body surfaces, including the mouth and nose.)	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Kirkpatrick DT (2005a)

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

In a key study, the acute inhalation toxicity of dimethyl disulphide (DMDS, purity 99.72%) was evaluated in single 4-hour, whole-body exposure study in male and female Sprague-Dawley rats performed in compliance with the EPA/OPPTS Guideline 870.1300 (1998) and the OECD Guidelines #403 (Kirkpatrick, 2005). The animals were exposed to concentrations of 847, 1188, 1308 and 1650 ppm then observed for 14 days for mortality, clinical signs, effect on body weight and then necropsied. Mortality following 4 hour whole body

exposure to DMDS was 0 animals at 847 ppm, 4/10 at 1188 ppm, 4/10 at 1308 ppm and 9/10 at 1650 ppm. All deaths occurred during exposure or immediately following exposure. Toxicologically relevant pharmacotoxic signs consisted of rales in the 1188 and 1650 ppm groups, decreased defecation in the 1188 ppm group and dried material on various body surfaces, including the mouth and nose for the surviving 1650 ppm female. Detailed clinical observations immediately following exposure consisted of salivation in the 1188, 1308 and 1650 ppm group males and/or females, tremors in the 847 and 1308 ppm group females, low arousal in the 847, 1188 and 1308 ppm group females, ataxia and impaired mobility in the 1188 ppm group females, hunched gait in the 1308 and 1650 ppm group females, and increased difficulty in removing from cage/handling in females in all groups. Over the course of the 8-hour observation interval, females in all groups had tremors, low arousal levels and were harder to remove from their cages and handle. Hunched gait was noted in the 1188 and/or 1308 ppm groups throughout the 8-hour period. Slight body weight losses (2 to 7 grams) were noted for one or more females each in the 847, 1188, 1308 ppm groups during the study. All surviving animals met or surpassed their initial (study day 0) body weight by study day 14. Dark red discolouration of the lungs was noted for animals found dead during or immediately following exposure in the 1308 and 1650 ppm groups. One male in the 1308 ppm group also had lungs that did not fully collapse at necropsy. There were no gross findings for any of the mortalities in the 1188 ppm group or for the surviving animals at the scheduled necropsy. Based on the results of this study, the LC50 of DMDS was 1310 ppm (with 95% confidence limits of 1167-1471 ppm) equivalent to 5.05 mg/L (with 95% confidence limits of 4.49-5.66 mg/l).

10.3.2 Comparison with the CLP criteria

The LC_{50} of DMDS as vapor was 1310 ppm (with 95% confidence limits of 1167-1471 ppm) equivalent to 5.05 mg/L (with 95% confidence limits of 4.49-5.66 mg/l).

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

In accordance with Regulation (EC) No 1272/2008, as the 4-hour inhalation LC_{50} is between 2.0 and 10.0 mg vapour/L, DMDS shall be classified as Acute Tox. 3 (Hazard statement: H331; Toxic if inhaled).

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral route

Rats

1. Dimethyl disulphide (DMDS) was tested for acute oral toxicity in Sprague-Dawley rats (6 females per dose), according to the EU Method B.1 in a GLP-compliant study (Pelcot, 2010). DMDS was administered orally a dose of 300 mg/kg bw in females.

No mortality was observed. The oral LD_{50} was found to be above 300 mg/kg bw for female rats.

2. DMDS was tested for acute oral toxicity in Sprague-Dawley rats (5 males and 5 females per dose), according to the EU Method B.1 in a GLP-compliant study (Lombard, 1986). DMDS was administered orally doses from 100 up to 500 mg/kg bw in males and females.

The oral calculated LD_{50} was established at 385 mg/kg bw combined for male and female rats which and it was within the range of values for classification in acute toxicity category 4 ($300 < LD_{50} \leq 2000$).

3. DMDS was tested for acute oral toxicity in Wistar rats (3 females given 501 mg/kg bw), according to the OECD TG 423 (with the following deviation; the observation period was terminated 6 days after the treatment) in a GLP-compliant study (Gilotti, 2006).

The incidence of mortality was 2/3 animals. The oral LD_{50} was therefore < 501 mg/kg bw for female rats.

4. DMDS was tested for acute oral toxicity in Wistar rats (5 males and 5 females per dose) using only two dose levels: 56 mg/kg bw for both sexes and 500 mg/kg bw for females only), according to the OECD TG 423 (with the following deviation; the observation period was terminated 6 days after the treatment) in a GLP-compliant study (Gilotti, 2007).

No mortality was observed at 56 mg/kg bw and all female rats died at 500 mg/kg bw. The oral LD_{50} was therefore above 56 and below 500 mg/kg bw for male and female rats.

5. The two additional studies (Shapiro, 1985a and 1985b) were disregarded due to contamination of tested DMDS with methyl mercaptan as indicated by the DS during the public consultation. Contaminated DMDS was tested for acute oral toxicity in Wistar rats (5 males and 5 females per dose), according to the EPA 40 CFR 163.81-1 in a GLP-compliant study (Shapiro, 1985a and 1985b). The oral LD_{50} was found to be between 125 and 250 mg/kg bw for male and female rats.

6. An additional study (Yasso, 2015) was submitted by the DS during the public consultation. In order to address the requirements of the Chinese authorities for the registration of DMDS as a PPP in China, a new acute oral toxicity study in male and female rats (Yasso, 2015) was performed by the Arkema's subsidiary in the USA on the technical DMDS (purity 99.89%). This study was performed following the Chinese guideline, which is comparable to the former OECD TG 401. The LD_{50} was 415 mg/kg bw with 95% confidence limits: 207 to 833 mg/kg bw for male rats and 750 mg/kg bw with 95% confidence limits: 362 to 1552 mg/kg bw for female rats. The RSS of this study is displayed in Appendix 2 to the RCOM.

Based on these data the Dossier Submitter (DS; Arkema France) proposed classification of DMDS for the oral route as Acute Tox. 4; H302.

Dermal route

1. DMDS was tested for acute dermal toxicity in Wistar rats (5 males and 5 females per dose), according to the EPA OPPTS 870.1200 guideline in a GLP-compliant study (Gilotti, 2007). No mortality was observed. The LD_{50} for both male and female rats was therefore above 5000 mg/kg bw.

2. In four supporting studies DMDS was tested for acute dermal toxicity in New Zealand White rabbits (5 males and 5 females per dose) (Shapiro, 1986 and 1985c; Sheppard, 1985; Haynes, 1988). No mortality was observed. In these studies the LD_{50} for both male

and female rabbits was therefore above 2000 mg/kg bw.

The DS concluded that no classification was warranted for DMDS since the acute dermal LD_{50} of DMDS was higher than 2000 mg/kg in rats and rabbits in all conducted acute dermal toxicity studies.

Inhalation route

In an acute inhalation study DMDS was tested in Sprague-Dawley rats (5 males and 5 females per dose) via whole body vapour exposure, according to the EPA OPPTS 870.1300/ OECD TG 403 (Kirkpatrick, 2005a). The established LC_{50} of DMDS was 5.05 mg/L air/4h (1310 ppm) with CI: 4.49 - 5.66 mg/L air/4h for rats. The DS proposed classification as Acute Tox. 3; H331 for the inhalation route.

Comments received during public consultation

3 MSCAs and one industrial organisation supported classification of DMDS by inhalation as Acute Tox. 3; H331.

Two MSCAs proposed classification of DMDS by the oral route as Acute Tox. 3; H301 based on Shapiro (1985a), in which the LD_{50} was between 125-188 mg/kg bw for males and between 188 and 250 mg/kg bw for females. According to the DS, DMDS (purity at least 98%) tested in this study was produced in a plant in the USA and contained a higher level of a toxicologically relevant impurity (methyl mercaptan) and therefore did not correspond to the DMDS (purity 99.88%) manufactured in France. The DS informed that the study by Shapiro (1985a) was included in the CLH report for completeness only, but that it should not be considered for establishing the classification of the substance.

Assessment and comparison with the classification criteria

Oral route

In the study of Lombard (1986) the LD_{50} values were within the range of the classification criteria for acute oral toxicity category 4 ($300 < LD_{50} \leq 2000$ mg/kg bw). In the Pelcot study (2010) the LD_{50} was above 300 mg/kg bw. In the study by Gilotti (2006) only a dose of 501 mg/kg bw was tested (mortality was 2/3 animals), fitting within the range of values for category 4, but a more severe classification category could not be excluded. In the second Gilotti study (2007), the oral LD_{50} was above 56 and below 500 mg/kg bw. Based on the results of both Gilotti studies, the LD_{50} was within the range of values for acute toxicity category 3 ($50 < LD_{50} \leq 300$ mg/kg bw/day) or 4 ($300 < LD_{50} \leq 2000$ mg/kg bw/day), therefore these studies were inconclusive.

In two studies by Shapiro (1985a and 1985b), the LD_{50} values were within a range of classification criteria for the acute toxicity category 3 ($50 < LD_{50} \leq 300$ mg/kg bw/day). These studies were conducted with DMDS (purity 98%) contaminated with up to 1% methyl mercaptan (EC no.: 200-822-1). The current CLH of methyl mercaptan for acute toxicity in Annex VI Table 3.1 (CLP) is Acute Tox. 3*; H331, but it is not classified for acute oral toxicity. In addition, methyl mercaptan has been predicted to be one of the major metabolites of DMDS. Therefore, RAC concludes that the presence of methyl mercaptan as an impurity at such a low concentration does not affect the acute oral

toxicity profile of DMDS, and the Shapiro studies are considered valid.

In the additional study (Yasso, 2015), the LD_{50} for male and female rats were found within the range for classification in acute toxicity category 4 (300 < LD_{50} ≤ 2000 mg/kg bw/day).

Taking into account the results of above studies, RAC considers that classification of DMDS in category 3 for acute oral toxicity is warranted. The lowest LD_{50} of 190 mg/kg bw determined in Shapiro (1985a) study for male and female rats is accepted by RAC as the ATE value for determining acute oral toxicity of mixtures containing DMDS.

Dermal route

The dermal LD_{50} values in all studies were above the classification criteria for acute dermal toxicity (LD_{50} ≤ 2000 mg/kg bw/day).

Inhalation route

The 4-hour LC_{50} via the inhalation route was 5.05 mg/L, which is within the range of values for classification in category 3 (2.0 < LC_{50} ≤ 10.0 mg vapour/L). Based on this LC_{50} value, RAC concludes that the ATE value for acute inhalation toxicity is also 5 mg/L.

Taking into account the data presented on acute toxicity by oral, inhalation and dermal routes, RAC is of the opinion that DMDS meets the classification criteria for:

- **Acute Tox 3; H301 (Toxic if swallowed)** and
- **Acute Tox. 3; H331 (Toxic if inhaled).**

No classification is warranted for dermal route, as proposed by DS.

10.4 Skin corrosion/irritation

Table 14: Summary table of animal studies on skin corrosion/irritation

Method	Results	Remarks	Reference
rabbit (New Zealand White) Coverage: semiocclusive (shaved) Vehicle: unchanged (no vehicle) OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	slightly irritating (not classified) Erythema score: 1.78 of max. 4 (mean) (Time point: 24+48+72 h) (not fully reversible within: 72 h) Edema score: 1.22 of max. 4 (mean) (Time point: 24+48+72 h) (not fully reversible within: 72h)	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Guillot JP (1985a)
rabbit (New Zealand White)	slightly irritating (Not classified) Erythema score:	4 (not assignable) weight of evidence	Shapiro R (1985d)

Method	Results	Remarks	Reference
Coverage: occlusive (shaved) Vehicle: unchanged (no vehicle) EPA 40 CFR 163.81-5	1.03 of max. 4 (mean) (Time point: 24+48+72 h) (fully reversible within: 10 days) Edema score: 0.11 of max. 4 (mean) (Time point: 24+48+72 h) (fully reversible within: 48 h)	experimental result **Test material (EC name): dimethyl disulphide**	

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

The acute dermal irritation of dimethyl disulphide (DMDS) was evaluated in rabbits according to OECD 404 guideline (Guillot, 1985). Dimethyl disulphide (purity 98.98%) was applied undiluted to the skin of 6 New-Zealand White albino rabbits and held in contact for 4 hours by means of a semi-occlusive dressing. Mean scores over 24, 48 and 72 hours were 1.78 and 1.22 for erythema and for edema, respectively. DMDS was considered as slightly irritating for the skin.

In another study performed according to EPA 40 CFR 163.81-5 guideline (Shapiro, 1985). DMDS (purity unknown) was applied undiluted to the intact skin of 6 New-Zealand White albino rabbits and held in contact for 24 hours by means of an occlusive dressing. For the shaved skin, mean scores over 24, 48 and 72 hours were 1.03 and 0.11 for erythema and for edema, respectively. All lesions were reversible within 10 days. DMDS was considered as slightly irritating for the skin.

10.4.2 Comparison with the CLP criteria

DMDS induced a slight skin irritation. In a key study (Gillot, 1985), the overall mean scores for the 6 rabbits (over 24, 48, and 72 hours) for erythema and eschar formation and oedema were 1.78 and 1.22 respectively. All individual mean scores for erythema and oeadema were below 2.3 (1.33-2.0 for erythema and 1-1.67 for oedema). The slight skin irritation observed in a second study (Shapiro, 1985) was reversible within 10 days.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

In accordance with Regulation (EC) No 1272/2008, DMDS was slightly irritant but does not merit classification as a skin irritant.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The skin irritation potential of DMDS was assessed in a study carried out according to OECD TG 404 (Guillot, 1985a) in 6 New Zealand White rabbits.

Mean scores over 24, 48 and 72 hours were 1.78 and 1.22 for erythema and oedema, respectively. Under these experimental conditions, DMDS was slightly irritating when applied topically to rabbits. These lesions were not fully reversible within 72 h, but observations on skin reactions were not reported beyond this time point.

Individual and mean skin irritation scores (Guillot, 1985a):

	Erythema/Oedema					
Animal No:	**11000**	**11001**	**11002**	**11005**	**11010**	**11042**
after 24 h	2 / 2	2* / 1	2* / 2	2* / 2	2 / 2	2* / 2
after 48 h	2 / 1	2* / 1	2* / 2	1* / 1	2 / 1	2* / 2
after 72 h	1 / 0	2* / 1	2* / 1	1* / 0	1 / 0	2* / 1
mean score 24/48/72 h	1.67 / 1	2 / 1	2 / 1.67	1.33 / 1	1.67 / 1	2/1.67

*= reaction is seen beyond the area of application

In another study performed according to the EPA 40 CFR 163.81-5 guideline (Shapiro, 1985), undiluted DMDS was applied to the intact skin of 6 New Zealand rabbits. Mean scores over 24, 48 and 72 hours were 1.03 and 0.11 for erythema and oedema, respectively. All lesions were reversible within 10 days.

DMDS was considered slightly irritating to the skin, but the DS proposed no classification for skin corrosion/irritation since the CLP criteria were not met.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Since in the acceptable study, Guillot (1985a), the CLP criteria for skin irritation were not fulfilled, RAC considers that DMDS does not warrant classification for skin corrosion/irritation. Although the reversibility of effects was not examined for the 14 day period recommended in the TG, the findings are assumed to be reversible, since their intensity was not very high (below criteria for classification) and in the study of Shapiro (1985) similar skin responses were fully reversible within 10 days.

The results of the study performed according to the EPA 40 CFR 163.81-5 guideline by Shapiro (1985) is in line with the results of Guillot (1985a). In this study, the mean scores from skin gradings at 24, 48 and 72 hours after patch removal did not reach 2.3 or higher for erythema/eschar or for oedema in any animal.

Since the slightly irritating skin effects observed in both studies did not meet the classification criteria (≥ 2.3 for oedema or erythema), and although the reversibility of the effects in Guillot (1985a) was not investigated, because similar skin responses in the study by Shapiro (1985) were fully reversible in 10 days, RAC is of the opinion that **no classification is warranted for skin irritation**.

10.5 Serious eye damage/eye irritation

Table 15: Summary table of animal studies on serious eye damage/eye irritation

Method	Results	Remarks	Reference
rabbit (New Zealand	Category 2 (irritating to eyes)	1 (reliable without	Guillot JP (1985b)

Method	Results	Remarks	Reference
White) Vehicle: unchanged (no vehicle) OECD Guideline 405 (Acute Eye Irritation / Corrosion)	Cornea score: 0.83 of max. 4 (mean) (Time point: 24+48+72 h) (not fully reversible within: 72 h) Iris score: 1 of max. 2 (mean) (Time point: 24+48+72 h) (not fully reversible within: 72 h) Conjunctivae score: 1.33 of max. 3 (mean) (Time point: 24+48+72 h) (not fully reversible within: 72 h) Chemosis score: 1.89 of max. 4 (mean) (Time point: 24+48+72 h) (not fully reversible within: 72 h)	restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	
rabbit (New Zealand White) Vehicle: unchanged (no vehicle) EPA-40 CFR 163-81-4	slightly irritating Cornea score: 0.33 of max. 4 (mean) (Time point: 24+48+72 h) (fully reversible within: 7 days) 0 of max. 4 (mean) (Time point: 24+48+72h) Iris score: 0 of max. 2 (mean) (Time point: 24+48+72 h) 0 of max. 2 (mean) (Time point: 24+48+72h) Conjunctivae score: 1.32 of max. 3 (mean) (Time point: 24+48+72 h) (fully reversible within: 7 days) 0.66 of max. 3 (mean) (Time point: 24+48+72 h) (fully reversible within: 72 h) Chemosis score: 1 of max. 4 (mean) (Time point: 24+48+72 h) (fully reversible within: 7 days) 0.44 of max. 4 (mean) (Time point: 24+48+72h) (fully reversible within: 72 h)	4 (not assignable) weight of evidence experimental result **Test material (EC name): dimethyl disulphide**	Shapiro R (1985e)

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

In a key study (Guillot, 1985), 0.1mL of undiluted DMDS (purity 98.98%) was instilled into the conjunctival sac of the eye of 6 rabbits. Mean scores for the 6 rabbits calculated over 24, 48 and 72 hours were 1.89 for chemosis, 1.33 for redness of the conjunctiva, 1.00 for iris lesions and 0.83 for corneal opacity. Dimethyl disulphide was considered as irritant when administered by ocular route to rabbits.

In a supporting study (Shapiro, 1985), 0.1mL of undiluted DMDS (purity unknown) was instilled into the conjunctival sac of the eye of 9 rabbits. The eyes were not rinced for 6 rabbits or rinsed after 20-30 sec. for 3 rabbits after administration. Ocular reactions were observed approximately 1 hour, 24, 48 and 72 hours after the administration. Corneal opacity was observed in the unwashed eyes of 2 rabbits. The opacity was transient and disappeared by 7 days. Transient hyperemia and chemosis were also observed in all rabbits (washed and unwashed eyes). These conditions disappeared between 24 and 96 hours post instillation. No iridial damage was noted in any of the rabbits. Mean scores calculated over 24, 48 and 72 hours for the 6 unwashed eyes were 1.0 for chemosis, 1.33 for redness of the conjunctiva, 0.0 for iris lesions and 0.33 for corneal opacity. Under these experimental conditions, DMDS was considered slightly irritating to the eyes.

10.5.2 Comparison with the CLP criteria

The mean score of 6 rabbits for iritis over 24, 48 and 72 hours obtained in the key study was equal to the threshold value (iris >= 1) for classification as irritant.

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

In accordance with Regulation (EC) No 1272/2008, DMDS was classified as irritating to eyes 2 (Hazard statement H319; Causes serious eye irritation).

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye damage/irritation potential of DMDS was assessed according to OECD TG 405 (Guillot, 1985b), but with the following deviation; the observation time after exposure was shortened to 3 days.

Mean scores for the 6 rabbits calculated over 24, 48 and 72 hours were 1.89 for chemosis, 1.33 for redness of the conjunctiva, 1.00 for iris lesions and 0.83 for corneal opacity. All lesions were not fully reversible within 72 h, but observations were not recorded after that time point.

Individual and mean eye irritation scores:

Reading time	Rabbit no	Cornea opacity	Iritis	Conjunctivae	
				Redness	Chemosis
1h	1	0	1	2	2
	2	0	1	1	2
	3	0	1	1	2
	4	2	1	1	2
	5	2	1	2	2
	6	2	1	2	2
24h	1	2	1	2	2
	2	2	1	2	2

	3	0	1	1	2
	4	2	1	1	2
	5	2	1	2	2
	6	2	1	1	2
48h	1	0	1	2	2
	2	2	1	2	2
	3	0	1	1	2
	4	0	1	1	2
	5	1	1	2	2
	6	0	1	1	2
72h	1	0	1	2	2
	2	1	1	1	2
	3	0	1	0	1
	4	0	1	1	2
	5	1	1	1	1
	6	0	1	1	2
mean score 24/48/72 h	1	0.67	1	2	2
	2	1.67	1	1.67	2
	3	0	1	0.67	1.67
	4	0.67	1	1	2
	5	1.33	1	1.67	1.67
	6	0.67	1	1	2
mean score 24/48/72 h (all animals)		0.83	1	1.33	1.89

In a supporting study (Shapiro, 1985e), the mean scores calculated over 24, 48 and 72 hours for the 6 unwashed eyes were 1.67; 1.67; 0.66; 0.33; 0.33; 1.33 for chemosis, 0.67; 0; 0.67; 0; 0; 0.33 for redness of the conjunctiva, 0; 0; 0; 0; 0; 0 for iris lesions and 0; 0; 0; 0,33; 0; 1.67 for corneal opacity. All lesions were fully reversible within 7 days.

An additional study was submitted by the DS during the public consultation in response to a comment received. For the registration of DMDS in China as a PPP, an eye irritation study in rabbits (Hall, 2015) had been performed on the technical DMDS (purity 99.89%) by Arkema's subsidiary in the USA. This study was performed following the Chinese guideline, which is comparable to the OECD TG 405. Over 24, 48 and 72h, corneal opacity and iris scores were 0 for four rabbits, conjunctival scores were 0.66 for two rabbits, 1.0 for the third rabbit and 0.33 for the fourth rabbit. Chemosis scores were 0.33 for two rabbits and 0 for two rabbits. All effects were reversed within 48-72 hours. The RSS of this study is displayed in Appendix 3 of the RCOM.

The DS proposed to classify DMDS as Eye Irrit. 2; H319 (Causes serious eye irritation).

Comments received during public consultation

Two MSCAs supported classification of DMDS as Eye Irrit. 2; H319 (Causes serious eye irritation).

One MSCA proposed a classification of DMDS with Eye Dam. 1; H318 based on the Guillot study, because the effects were not assessed after 72 hours and therefore the reversibility of the effects could not be assessed.

Assessment and comparison with the classification criteria

In the key study (Guillot, 1985b), effects meeting the CLP criteria for eye irritation category 2 (a mean score of ≥1 for iritis and a mean score of ≥2 for conjunctival chemosis calculated following grading at 24, 48 and 72 hours after installation of the test material at least in 2 of 3 tested animals) were observed in tested animals. The reversibility of the effects was not assessed in this study, but the value of individual eye irritation scores had a declining trend over time. In supporting studies by Shapiro (1985e) and Hall (2015), the effects had fully reversed in 7 days and 48-72 hours, respectively, although the mean scores observed in these studies did not meet the CLP criteria for Eye Irrit. 2; H319.

Considering all the available evidence, RAC concludes that DMDS warrants **classification as Eye Irrit. 2, H319 (Causes serious eye irritation)**.

10.6 Respiratory sensitisation

No data are available for that specific hazard class.

10.7 Skin sensitisation

Table 16: Summary table of animal studies on skin sensitisation

Method	Results	Remarks	Reference
mouse (CBA) female Local lymph node assay OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay) EU Method B.42 (Skin Sensitisation: Local Lymph Node Assay)	sensitising (category 1B) Stimulation index: The EC3 value is approximately 2.50%.	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Rokh N (2012)

Table 17: Summary table of other studies relevant for skin sensitisation

Method	Results	Remarks	Reference
in vitro study Direct peptide binding assay Binding to Cys and Lys model peptides in chemico (Bauch C. et al 2011)	Peptide binding	1 (reliable without restriction) supporting study experimental result **Test material**	BASF (2013a)

Method	Results	Remarks	Reference
		(EC name): dimethyl disulphide	
in vitro study U937 cell line (human dentritic-like cell line) Dentritic cell activation assay CD 86 expression in dentritic cell line as marker for stimulation (Myeloid U937 Skin Sensitization Test, MUSST)	activation of dentritic cells in vitro	1 (reliable without restriction) supporting study experimental result **Test material (EC name): dimethyl disulphide**	BASF (2013b)
in vitro study LuSens cells (derived from human keratinocyte cell line HaCaT) in-vitro keratinocyte activation assay (LuSens) The LuSens assay is an in vitro method for the identification of keratinocyte activating substances using the genetically modified keratinocytes (LuSens, Bauch et al. 2012). lt employs the reporter gene for luciferase under the control of an antioxidant response element and hence monitors Nrf-2 transcription factor activity. The endpoint measurement is the up-regulation of the luciferase activity after 48 hour incubation with test substances. This up-regulation is an indicator for the activation of the Keap1/Nrf2/ARE signaling pathway (Ade et al. 2009, Natsch 2012, Natsch & Emter 2008, Vandebriel et al. 2010).	no induction of antioxidant response genes in a keratinocyte cell line	1 (reliable without restriction) supporting study experimental result **Test material (EC name): dimethyl disulphide**	BASF (2013c)

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

In vivo studies

An OECD test guideline no. 429 compliant GLP study was conducted to investigate the potential of Dimethyl disulphide (DMDS, purity 99.8%) to induce delayed contact hypersensitivity using the murine Local Lymph Node Assay (LLNA) (Rokh, 2012). On the basis of a preliminary study, 2.5, 5, 10, 25 and 50% DMDS concentrations were selected and the vehicle was 4/1 acetone/olive oil. A positive control group (α-hexylcinnamaldehyde (HCA)) was included in the study for validation purposes. During the induction phase,

DMDS, vehicle or HCA was applied over the ears (25 µL per ear) for 3 consecutive days (days 1, 2 and 3) and after 2 days of resting, the proliferation of lymphocytes in the lymph node draining the application site was measured by incorporation of tritiated methyl thymidine (day 6). The obtained values were used to calculate Stimulation Indices (SI). The irritant potential of the test item was assessed in parallel by measurement of ear thickness on days 1, 2, 3 and 6.

In the preliminary test one animal treated topically with 100% DMDS was found dead on day 3. The cause of death was not established. In the main test DMDS had no effect on body weight, was not associated with clinical signs or local reactions and did not increased ear thickness.

The acceptance criteria for the positive control (Stimulation index ≥3) was met, thus the study was considered valid. In view of the absence of a dose-response relationship a second counting was performed to check the disintegration count results; the values obtained at the first count were confirmed. A significant lymphoproliferation (SI > 3) was noted at DMDS concentrations of 2.5, 25 and 50%. Therefore, despite the absence of a dose-response relationship and in the absence of local irritation, the significant lymphoproliferative responses observed were attributed to delayed contact hypersensitivity. The EC3value is approximately 2.5%.

The results for DMDS are shown in the table below:

Treatment	Concentration (%)	Irritation level	Stimulation Index (SI)
DMDS	2.5	non-irritant	2.99 / 3.38
DMDS	5	non-irritant	2.40 / 2.40
DMDS	10	non-irritant	1.90 / 1.85
DMDS	25	non-irritant	3.30 / 3.58
DMDS	50	non-irritant	4.75 / 4.77
HCA	25	-	20.98 / 19.98

- not recorded

... /... first counting / second counting

It may therefore be concluded that under the experimental conditions of this study, DMDS (purity 99.8%) induced delayed contact hypersensitivity in the murine Local Lymph Node Assay. According to the EC3 value obtained (approximately 2.5%), the test item should be considered as a moderate sensitiser.

In vitro studies

A combination of several in vitro methods addressing key steps of the adverse outcome pathway (AOP) for skin sensitization as defined by OECD, has been conducted to assess the skin sensitizing potential of DMDS.

• protein reactivity (DPRA),

• activation of keratinocytes (LuSens), and

• activation of dendritic cells (MUSST).

The results of the individual studies are summarized and evaluated to predict the presence or absence of skin sensitizing potential of DMDS. The combination of test methods and the evaluation of their results has been evaluated and published by Bauch et al., 2012. Based on the performance standards of the OECD test guideline no. 429 (Local Lymph Node Assay, LLNA, OECD 20103), the evaluation based on the DPRA, LuSens and MUSST methods yields an overall accuracy of 95% compared to results in humans (for comparison: for the same data set the LLNA yielded an overall accuracy of 86%). A skin sensitizing (quantitative) potency assessment using the reported results was not validated at the present time. Based on the results summarized and applying the evaluation criteria, DMDS is predicted to be a skin sensitizer.

- in vitro DPRA

The reactivity of DMDS towards synthetic cysteine (C) - or lysine (K) -containing peptides was evaluated in the Direct Peptide Reactivity Assay (DPRA) (BASF, 2013a). For this purpose the test substance was incubated with synthetic peptides for ca. 24 hours at room temperature and the remaining non-depleted peptide concentrations were determined by high performance liquid chromatography (HPLC) with gradient elution and UV-detection at 220 nm. The test substance was dissolved at a 100 mM concentration in acetonitrile. Three samples of the test substance were incubated with each peptide in ratios of 1:10 (for C-peptide) or 1:50 (for K-peptide). Additionally triplicates of the concurrent vehicle control (= NC) were incubated with the peptides. Further, a co-elution control was performed in order to detect possible interference of the test substance with the peptides. The samples consisted of the test substance, vehicle and the respective peptide buffer but without peptide. Moreover the samples were analyzed by measuring UV absorbance at 258 nm and the area ratio 220 / 258 was calculated as a measure of peak purity.

The following results were obtained in the DPRA:

The test substance was solved in acetonitrile. The samples of the test substance with the peptides were solutions. Visual observation after the 24-hour incubation time did not reveal precipitates in all samples of the test substance with both peptides.

The mean C-peptide depletion, caused by the test substance was determined to be 96.80%.

The mean K-peptide depletion, caused by the test substance was determined to be 0.76%.

Thus, the mean peptide depletion was calculated to be 48.78%.

No co-elution of test substance and peptides was noticed.

Based on the observed results and applying the prediction model proposed in Gerberick et. al (2007) and cited in chapter 3.10 it was concluded that DMDS shows a high chemical reactivity in the DPRA under the test conditions chosen.

- in vitro MUSST

The potential of test substance DMDS to induce the cell membrane marker CD86 expression was evaluated in the Myeloid U937 Skin Sensitization Test (MUSST) (BASF, 2013b). For this purpose the test substance was incubated with human pro-monocytic cell line U937 for ca. 48 hours at 37°C and membrane marker expression was measured by flow cytometry. In order to determine the concentrations suitable for the main experiment a pre-test (experimental conduct in accordance with GLP but without a GLP status) was performed. Cells were exposed to 9 concentrations of the test substance (0.5 µg/mL up to 2000 µg/mL) and cytotoxicity was determined thereafter by propidium iodide (PI) intercalation into the DNA. The CV75 value (= estimated concentration that affords 75% cell viability) was determined by linear regression from the concentration response curve. In the main test after 48 hour exposure U937 cells were stained with FITC labeled antihuman-CD86 antibody and propidium iodide, the fluorescence intensity was analyzed using flow cytometry. A total of 2 valid experiments were performed.

The test substance was soluble in DMSO (400 x stock solutions) and soluble in 0.25% DMSO (final concentrations).

After 48 hours precipitates were not noticed in any concentration. After 48 hours of exposure to test substance DMDS, CD86 expression was induced in U937 cells affording at least 70% viability in two independent experiments. From this it has to be concluded that test substance DMDS does induce dendritic cell activation.

- ARE Reporter Assay - LuSens

The keratinocyte activating potential of test substance DMDS was evaluated in the LuSens assay (BASF, 2013c). For this purpose the test substance was incubated with a luciferase reporter cell line (LuSens cells) for ca. 48 hours at 37°C and antioxidant response element (ARE) dependent luciferase activity was measured in a luminometer. In order to determine the concentrations suitable for the main experiment a pre-test was performed. Cells were exposed to 9 concentrations of the test substance and cytotoxicity was determined thereafter by MTT assay. The CV75 value (= estimated concentration that affords 75% cell viability) was

determined by linear regression from the concentration response curve. In the main test luciferase activity was measured after 48 hour exposure. In parallel a MTT assay was performed to assess cytotoxicity of the test substance. A total of 2 valid experiments were performed. The following results were observed:

The test substance (100 x stock solutions) was soluble in DMSO and soluble in 1% DMSO (final concentrations). After 48 hours precipitates were not noticed in any concentration. After 48 hours of exposure to DMDS luciferase activity in LuSens cells was not induced affording at least 70% viability in at least two independent experiments. From this it has to be concluded that DMDS does not have a keratinocyte activating potential.

10.7.2 Comparison with the CLP criteria

In a test performed following the LLNA's method (Rokh, 2012), dimethyl disulphide induces skin sensitiziation in mice with an EC3 of 2.5%.

10.7.3 Conclusion on classification and labelling for skin sensitisation

In accordance with Regulation (EC) No 1272/2008, as EC3 value is > 2 %, DMDS was classified as skin sensitizer category 1B (Hazard statement H317; May cause an allergic skin reaction).

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The dossier submitter had included 4 studies to evaluate the skin sensitising potential of DMDS; one *in vivo* Local Lymph Node Assay (LLNA) and three *in vitro* tests addressing key events in the AOP for skin sensitisation (DPRA, LuSens, MUSST).

In the LLNA (Rokh, 2012), conducted according to OECD TG 429 (EU Method B.42), the potential of DMDS (purity 99.8%) to cause skin sensitisation was investigated using the following DMDS concentrations: 2.5, 5, 10, 25 and 50%. The vehicle was acetone/olive oil in proportion 4/1. The positive control group (α-hexyl cinnamic aldehyde (HCA)) was included in the study for validation purposes. During the induction phase, DMDS, vehicle or HCA was applied over the ears (25 µL per ear) for 3 consecutive days (days 1, 2 and 3), and after 2 days of resting, the proliferation of lymphocytes in the lymph node draining the application site was measured by incorporation of tritiated methyl thymidine (day 6). The obtained values were used to calculate Stimulation Indices (SI). The irritant potential of the test item was assessed in parallel by measurement of ear thickness on days 1, 2, 3 and 6. The results for DMDS are shown in the table below:

Treatment	Concentration (%)	Irritation level	Stimulation Index (SI) First counting/second counting
DMDS	2.5	non-irritant	2.99 / 3.38
DMDS	5	non-irritant	2.40 / 2.40
DMDS	10	non-irritant	1.90 / 1.85
DMDS	25	non-irritant	3.30 / 3.58
DMDS	50	non-irritant	4.75 / 4.77
HCA	25	not recorded	20.98 / 19.98

A significant lymphoproliferation (SI > 3) was noted at DMDS concentrations of 2.5, 25 and 50%, but not at concentrations of 5 and 10%. Despite the absence of a dose-response relationship and in the absence of local irritation, the significant responses observed were attributed to delayed contact hypersensitivity. The EC3 value was approximately 2.5%, thus slightly above the criterion for classification to sub-category Skin Sens. 1A (EC ≤2 %). However, lower concentrations were not tested.

In vitro sensitisation assays

DPRA: The reactivity of DMDS towards synthetic cysteine (C) - or lysine (K) -containing peptides was evaluated in the Direct Peptide Reactivity Assay (DPRA) (BASF, 2013a) in accordance with (Bauch *et al*., 2011; Gerberick *et al*., 2004, 2007) (the OECD TG 442C DPRA was adopted on 4 February 2015). For this purpose the test substance was incubated with synthetic peptides for *ca*. 24 hours at room temperature and the remaining non-depleted peptide concentrations were determined by high performance liquid chromatography (HPLC) with gradient elution and UV-detection at 220 nm. The mean Cys-peptide depletion, caused by the test substance was determined to be 96.80 % and the mean Lys-peptide depletion, caused by the test substance was determined to be 0.76 %. The mean peptide depletion by a test substance was calculated as the mean value of Cys containing peptide depletion and Lys-containing peptide depletion amounted to be 48.78%. According to the classification tree model, described by Gerberick *et al*. (2007), for substances with known molecular weight a highly reactive test substance (mean peptide depletion > 42.47 %) is predicted to be a strong sensitiser, and a moderately reactive test substance (22.62 % < mean peptide depletion < 42.47 %) a moderate sensitiser. Based on the observed results and applying the prediction model proposed in Gerberick *et al*. (2007), it was concluded that DMDS shows a high chemical reactivity in the DPRA under the test conditions chosen.

MUSST: The potential of DMDS to induce the cell membrane marker CD86 expression was evaluated in the Myeloid U937 Skin Sensitization Test (MUSST) (BASF, 2013b). For this purpose the test substance was incubated with the human pro-monocytic cell line U937 for *ca*. 48 hours at 37°C and membrane marker expression was measured by flow cytometry. The test substance was dissolved in DMSO (400 x stock solutions) and dissolved in 0.25% DMSO (final concentrations). After 48 hours precipitates were not noted at any concentration. After 48 hours of exposure to DMDS, CD86 expression was induced in U937 cells showing at least 70% viability in two independent experiments. From this it was concluded by the DS that DMDS did induce dendritic cell activation.

ARE Reporter Assay – LuSens: The keratinocyte activating potential of DMDS was evaluated in the LuSens assay (BASF, 2013c). DMDS was incubated with a luciferase reporter cell line (LuSens cells) for *ca*. 48 hours at 37°C and antioxidant response element (ARE)-dependent luciferase activity was measured in a luminometer. In order to determine the concentrations suitable for the main experiment a pre-test was performed. Cells were exposed to 9 concentrations of the test substance and cytotoxicity was determined thereafter by MTT assay. The CV75 value (= estimated concentration that shows 75% cell viability) was determined by linear regression from the concentration response curve. In the main test luciferase activity was measured after 48 hour exposure. In parallel, a MTT assay was performed to assess cytotoxicity of the test substance. A total of 2 valid experiments were performed. The following results were observed: after 48 hours precipitates were not observed at any concentration. After 48 hours of exposure to DMDS luciferase activity in LuSens cells was not induced showing at

least 70% viability in at least two independent experiments. From this it was concluded by the DS that DMDS did not have keratinocyte activating potential.

Based on results of the LLNA and taking the results of *in vitro* tests as supportive evidence, the DS proposed classification as Skin Sens. 1B; H317 (May cause allergic reactions).

Comments received during public consultation

One MSCA noted that the LLNA resulted in a SI >3 for 25% and 50% test concentrations and in an SI ≥ 3 for a 2.5% test concentration. For concentrations 5 and 10% the SI was below 3, and therefore there was no dose-response relationship. Since concentrations of DMDS below 2.5% were not tested in the LLNA, subcategory 1A could not be excluded (EC3 = 2.5%) and classification as Skin Sens. 1 was considered appropriate by the MSCA. The MSCA further noted that support for the skin sensitizing potential of DMDS is also derived from the positive outcome in two out of three *in vitro* tests. It was also remarked by the MSCA that the DS has omitted in the CLH report one study available at ECHA's dissemination site, namely a Buehler assay from 1985, which was negative.

Two other MSCAs noted that that there was not enough data for subcategorisation, since it could not be excluded that DMDS below concentration of 2% may induce SI ≥3. One MSCA commented that it was unclear whether the EC3 was above or below 2%, and therefore both options Skin Sens. 1 or 1B should be considered.

In its response the DS informed that the Buehler assay from 1985 was not reported in the CLH report because it was negative and therefore of limited value for the sub-categorisation of the sensitising potential of DMDS. In addition, the DS provided results of another LLNA assay, which was performed with a plant protection formulation (named Atomal13) containing 93.1% of DMDS (Watzinger, 2011). This study was not originally included in the CLH report. The results were as follows:

Treatment	Concentration (%)	Irritation level	Stimulation Index (SI)
Atomal13	2.5	non-irritant	0.91
Atomal13	5	non-irritant	1.07
Atomal13	10	non-irritant	0.79
Atomal13	25	non-irritant	3.46
Atomal13	50	non-irritant	4.10
HCA	25	-	4.83

A significant lymphoproliferation (SI > 3) was noted only at the concentrations of 25% and 50%, but not at concentrations of 2.5, 5 and 10 %. In the absence of local irritation, the significant lymphoproliferative responses observed were attributed to delayed contact hypersensitivity. The EC3 value was equal to 22.41%. Therefore, the SI ≥ 3 at 2.5% of DMDS in the Rokh (2012) study was considered questionable by the DS and not relevant for sub-categorisation. The SI observed at 25 and 50% in both studies were comparable and justified in the opinion of DS the classification of DMDS as Skin Sens. 1B.

Assessment and comparison with the classification criteria

In the current Guidance on the Application of CLP Criteria (point 3.4.2.2.2) it is noted that classification into sub-categories is only possible if the data are sufficient. Therefore, care should be taken when classifying substances into category 1B when category 1A cannot be excluded. In such cases classification into category 1 should be considered. This is particularly important if only data are available from certain tests showing a high response after exposure to a high concentration, but where lower concentrations which could show the presence of such effects at lower doses are absent.

In order to classify a substance into sub-category 1A in the Local lymph node assay, a value of EC3 should be ≤ 2 % while that for the subcategory 1B should be > 2 %. Therefore, in order to classify in sub-category 1B (if the EC3 is > 2 %), there is also a need for data demonstrating that DMDS at a concentration of ≤ 2 % will not induce an SI ≥ 3 and is therefore not meeting the CLP criteria for sub-category 1A.

In the LLNA of Rokh (2012), performed with DMDS (purity 99.8%) no significant stimulation of proliferation of cells was observed at concentration of the 5% – 10%, but at a concentration of 2.5% an SI of approx. 3 was noted in two countings. It is uncertain whether this SI at a concentration 2.5% was in fact induced by DMDS since it was not observed at the two higher concentrations 5 and 10%. However, no concentration of ≤ 2 % was tested and based on the result of this study, classification in category 1A cannot be excluded.

In the LLNA performed with a plant protection product (named Atomal13) containing 93.1% of DMDS (Watzinger, 2011), no significant stimulation of proliferation of cells was observed at 2.5% – 10% concentration of the formulation. The concentrations of DMDS in these trials were even slightly lower noting that its content in the formulation was 93.1%. However, it is noted that Atomal13 is not a substance, but a mixture, and therefore even though this study provides evidence that EC3 for this plant protection product is above 10%, the category 1A for DMDS cannot be excluded.

In addition to the LLNA, the results of three in chemico /*in vitro* tests and the Buehler assay were used in weight of evidence analysis to assess skin sensitising potential of DMDS.

The results of DPRA (BASF, 2013a) evaluating the peptide reactivity of DMDS, which is the first key event of the skin sensitisation AOP, demonstrated high reactivity of DMDS in this test system. At present, there are no established rules for assessment of skin sensitising potency based on results of the positive test, so the results are taken as supportive evidence for skin sensitising potential of DMDS.

The antioxidant response element (ARE) was not induced in the LuSens assay (BASF, 2013c). This assay evaluates a potential of the test substance to induce cyto-protective gene pathways in keratinocytes (the second event in the skin sensitisation AOP). The result of the study suggests that this pathway was not or was only slightly activated by DMDS which together with negative results in the Buehler assay indicates that skin sensitising potential of DMDS is not very high.

Induction of specific cell surface marker CD86 in the U937 cells in the Myeloid U937 Skin Sensitisation Test (MUSST) (BASF, 2013b) indicates that DMDS activates the dendritic cells, which is the third key event of the skin sensitisation AOP. The results of this *in vitro*

assay are taken as supportive evidence for skin sensitising potential of DMDS.

Taking into account this weight of evidence, RAC is of the opinion that DMDS warrants classification as **Skin Sens. 1; H317 (May cause allergic reactions)**.

10.8 Germ cell mutagenicity

Table 18: Summary table of mutagenicity/genotoxicity tests in vitro

Method	Results	Remarks	Reference
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) E. coli WP2 uvr A (met. act.: with and without) Test concentrations: The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate in the initial toxicity-mutation assay and 50, 150, 500, 1500 and 5000 µg per plate in the confirmatory mutagenicity assay. Positive control substance(s): With S9: 2-aminoanthracene, 1 µg/plate for all Salmonella strains, 10µg/plate for E. coli. Without S9: TA 98, 2-nitrofluorene (1 µg/plate); TA100 and TA 1535, sodium azide (1 µg/plate); TA 1537, 9-aminoacridine (75 µg/plate); E. coli, MMS (1000 µg/plate) OECD Guideline 471 (Bacterial Reverse Mutation Assay) EPA OPPTS 870.5100 - Bacterial Reverse Mutation Test (August 1998)	Evaluation of results: negative Test results: negative for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 and E. coli WP2 uvr A(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: no (Tested up to limit concentrations recommended by the test guideline) ; vehicle controls valid: yes; positive controls valid: yes	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Wagner VO (2007)
mammalian cell gene mutation assay (gene mutation) Chinese hamster Ovary (CHO) (met. act.: with and without) Test concentrations: First assay : 0.46, 1.37, 4.12, 12.3, 37.0, 111, 333 and 1,000 mg/l Second assay: 4.12, 12.3, 37.0, 74.1, 111, 333, 667 and 1,000 mg/l	Evaluation of results: negative without metabolic activation ambiguous with metabolic activation (positive at cytotoxic concentrations) Test results: ambiguous for Chinese hamster Ovary (CHO)(HGPRT assay on CHO cells) ; met. act.: with and	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Rutten AA (1990a)

Method	Results	Remarks	Reference
Positive control substance(s): Without S9: Ethylmethanesulfonate 0.2 ml/L . With S9: Dimethylnitrosamine 2 or 4 ml/L. OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	without ; cytotoxicity: yes (74.0-1000 µg/ml) ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes		
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium, other: TA98, TA 100, TA1535, TA1537, TA 1538 (met. act.: with and without) Test concentrations: (a) Preliminary cytotoxicity assay: Plate incorporation assay: 0, 5, 50, 500 and 5000 µg per plate were evaluated with and without S9 activation in all strains. A single plate was used, per dose, per condition. (b)Mutation assays: Plate incorporation assay: 50, 150, 500, 1500 and 5000 µg per plate were evaluated in triplicate in the presence and absence of S9 activation; all test strains were used. Positive control substance(s): With S9 mix: 2-Aminoanthracene (2 µg/plate) for TA 1535, TA 1537, TA 1538, TA 98 and TA 100. Without S9 mix: 2-nitrofluorene (10 µg/plate) for TA 1538 and TA98; 9-aminoacridine (20µg/plate) for TA 1537 and sodium azide (5µg/plate) for TA1535 and TA 100. EU Method B.13/14 (Mutagenicity - Reverse Mutation Test Using Bacteria) (1984)	Evaluation of results: negative Test results: negative for S. typhimurium, other: TA 1535, TA 1537, TA 1538, TA 98, TA 100(all strains/cell types tested) ; met. act.: with and without ; cytotoxicity: no (Tested up to limit concentrations recommended by the test guideline) ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes	1 (reliable without restriction) supporting study experimental result **Test material (EC name): dimethyl disulphide**	Jones E (1985)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium, other: TA 1535, TA 1537, TA 1538, TA 98, TA 100 (met. act.: with and without) Test concentrations: (a) Preliminary cytotoxicity assay: Plate incorporation assay: 0, 50,	Evaluation of results: negative Test results: negative for S. typhimurium, other:(TA 1535, TA 1537, TA 1538, TA 98, TA 100) ; met. act.: with and without ; cytotoxicity: no (Tested up to	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): dimethyl disulphide**	Barfknecht TR (1985)

Method	Results	Remarks	Reference
144, 500, 1444 and 5000 µg per plate were evaluated without S9 activation with strains TA100 and TA 1538. Two plate was used, per dose, per condition. (b) Mutation assays: Plate incorporation assay: 0, 50, 166, 500, 1666 and 5000 µg per plate were evaluated in triplicate in the presence and absence of S9 activation; all test strains were used. Positive control substance(s): With S-9 mix: 2-Aminoanthracene (5 µg/plate), TA 1535, TA 1537, TA 1538, TA 98 and TA 100. Without S-9 mix: 2-Nitrofluorene (5 µg/plate) TA 1538 and TA98, 9-Aminoacridine (150 µg/plate) TA 1537, sodium azide (10 µg/plate) TA 1535 and TA 100. OECD Guideline 471 (Bacterial Reverse Mutation Assay) (1983)	limit concentrations recommended by the test guideline) ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes		
in vitro mammalian chromosome aberration test (chromosome aberration) primary culture, other: Human Lymphocytes (met. act.: with and without) Test concentrations: 0; 3.7; 11.1; 33.3; 100; 300 µg/ml Positive control substance(s): Without S9: mitomycin C (MMC) 0.05 µg/mL. With S9: cyclophosphamide (CP) 25 µg/mL OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	Evaluation of results: ambiguous (positive at cytotoxic concentrations, both with and without metabolic activation) Test results: ambiguous for primary culture, other: Human Lymphocytes ; met. act.: with and without ; cytotoxicity: yes (clearly toxic at >= 300 µg/ml) ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	De Vogel N (1990)
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells in vitro (DNA damage and/or repair) primary culture, other: Rat hepatocytes (met. act.: not applicable) Test concentrations: * Cytotoxicity studies: - 1st study : 1 - 5 - 10 - 100 and	Evaluation of results: negative Test results: negative for primary culture, other: Rat hepatocytes ; met. act.: not applicable ; cytotoxicity: yes (>= 100 µg/ml. IC50 evaluated by LDH release: 98 µg/ml (2nd study)) ; vehicle controls valid: yes; negative controls valid:	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Bichet N (1990)

Method	Results	Remarks	Reference
200 µg/ml - 2nd study : 5 - 10 - 50 - 100 - 150 - 200 - 250 and 300 µg/ml * Genotoxicity studies: - 1st study: 1- 5 - 10- 50 - 100 and 200 µg/ml - 2nd study : 1 - 10 - 50 - 100 - 200 and 300 µg/ml Positive control substance(s): 7,12-DMBA (10 µM) and 2-aminofluorene (0.1 and 0.5 µM) OECD Guideline 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro)	yes; positive controls valid: yes		

Table 19: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method	Results	Remarks	Reference
micronucleus assay (chromosome aberration) rat (Sprague-Dawley) male/female inhalation: vapour 217, 421, and 825 ppm (analytical conc.) Positive control substance(s): - Cyclophosphamide - Justification for choice of positive control(s): - Route of administration: intraperitoneal injection 24 hours prior to bone marrow collection - Doses : 40 mg/kg OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test) EPA OPPTS 870.5395 (In Vivo Mammalian Cytogenics Tests: Erythrocyte Micronucleus Assay)	Evaluation of results: negative Test results: Genotoxicity: negative (male/female); toxicity: yes (Clinical signs and reductions in the ratio of PCEs to total erythrocytes) ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Weinberg JT (2007)
micronucleus assay (chromosome aberration) mouse (Swiss) male/female inhalation	Evaluation of results: negative Test results: Genotoxicity: negative (male/female); toxicity: yes (Mean numbers of PE/1000 E	1 (reliable without restriction) supporting study experimental result	Willems MI (1989)

Method	Results	Remarks	Reference
0 , 250 and 500 ppm (analytical conc.) Positive control substance(s): The positive control group (5/sex) was treated once intraperitoneally, 24 hours before sacrifice, with 1.5 mg Mitomycin C per kg body weight. OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	were slightly lower in mice exposed to 500 ppm) ; vehicle controls valid: yes; negative controls valid: not applicable; positive controls valid: yes	**Test material (EC name): dimethyl disulphide**	
unscheduled DNA synthesis (DNA damage and/or repair) rat (Wistar) male inhalation 0 and 500 ppm Positive control substance(s): The hepatocarcinogen 2-acetylaminofluorene (2 AAF: 50 mg/kg by gavage), was used as a positive control in the in vivo/in vitro DNA-repair assay and in the in vitro DNA-repair assay (2 AAF: final concentration of 10e-4 M in tissue culture medium). OECD guideline 482 (DNA Damage and Repair/Unscheduled DNA Synthesis in Mamnalian Cells in vitro) equivalent or similar to OECD Guideline 486 (Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo) (The study was performed before the implementation of the guideline)	Evaluation of results: negative Test results: Genotoxicity: negative (male); toxicity: yes (Increased GGT enzyme activities in plasma of rats exposed to 500 ppm) ; vehicle controls valid: yes; negative controls valid: not examined; positive controls valid: yes	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Rutten AA (1990b)

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

In vitro studies

In a key OECD 471 bacterial reverse mutation test (Wagner, 2007), dimethyl disulphide, was tested in the Bacterial Reverse Mutation Assay using Salmonella typhimurium tester strains TA98, TA100, TA1535 and TA1537 andEscherichia colitester strain WP2uvrAin the presence and absence of Aroclor-induced rat liver S9. The assay was performed using the plate incorporation method. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate in the initial toxicity-mutation assay and 50, 150, 500, 1500 and 5000 µg per plate in the confirmatory mutagenicity assay. No positive mutagenic response was observed.

Neither precipitate nor appreciable toxicity was observed. Dimethyl disulphide was concluded to be negative in the Bacterial Reverse Mutation Assay. In two other supporting OECD 471 bacterial reverse mutation tests, dimethyl disulphide was negative inSalmonellastrains TA 1535, TA 1537, TA 1538, TA 98, and TA 100, in the presence and absence of metabolic activation (Jones, 1985; Barfknecht, 1985).

In a key OECD 473 chromosome aberration study with human lymphocytes (De Vogel, 1990), dimethyl disulphide did not induce a statistically significant increase in the number of cells with structural chromosome aberrations at non-toxic concentrations (<= 100 µg/mL), both in the absence and in the presence of metabolic activation. At the very toxic concentration of 300 µg/mL, both in the absence and in the presence of metabolic activation, dimethyl disulphideinduced a statistically significant increase in the number of cells with structural chromosome aberrations.

In a key OECD 476 mammalian cell gene mutation assay (HGPRT) with CHO cells (Rutten, 1990), dimethyl disulphide(0.46, 1.37, 4.12, 12.3, 37.0, 74.0, 111, 333, 667 and 1000 µg/mL) did not increase the mutant frequency in the absence of metabolic activation. In the presence of a metabolic activation system, dimethyl disulphideinduced a slight increase in mutant frequency at several concentrations. These increases were not concentration-related or clearly reproducible. Dimethyl disulphideis highly toxic to CHO cells at a concentration range from 74-1,000 µg/mL. The actual concentrations of dimethyl disulphidein culture medium were much lower than the target concentrations. Recovery experiments showed that about 50% was lost directly on incubation (presumably by evaporation) and during incubation an additional 25% is lost (presumably reactions with constituents of the incubation). There was no conclusive evidence for a genotoxic effect of dimethyl disulphidein cultured CHO cells.

In a key OECD 482 DNA damage and repair assay (Bichet, 1990), dimethyl disulphide (1, 5, 10, 50, 100, 200 and 300 µg/mL; cytotoxic>100 µg/mL) was not genotoxic to rat hepatocytes in culture.

In vivo studies

In a key micronucleus assay performed following the OECD guideline # 474 and the OPPTS Guideline # 870.5395 dimethyl disulphide at concentrations of 217, 421 and 825 ppm did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythocytes in the bone marrow when male and female Sprague-Dawley rats were exposed to test article as a single, 4-hour, whole-body inhalation exposure (Weinberg, 2007).

In a supporting OECD 474 study, three groups of mice were exposed during 6 hours a day for 4 consecutive days to atmospheres containing 0, 250 and 500 ppm DMDS (Willems, 1989). Bone marrow cells were collected from the femur and examined for the presence of micronucleated poly- and normochromatic erythrocytes. Exposure to DMDS resulted in clear signs of toxicity at 250 ppm and 500 ppm, and 12 of the 20 mice of the 500 ppm group died. Mean numbers of polychromatic erythrocytes were slightly lower in mice exposed to 500 ppm DMDS, suggesting slight cytotoxic effects on bone marrow cells. There were no increases in the incidences of micronucleated erythrocytes attributable to DMDS exposure.

In a key OECD 482 unscheduled DNA synthesis test (Rutten, 1990), male rats were exposed by inhalation for a period of 4 h to 500 ppm dimethyl disulphide(maximally tolerated concentration). Immediately after exposure and after subsequent non-exposure periods of 16 and 24 h, animals were sacrificed for the isolation of hepatocytes. The DNA-repair activities were examined by autoradiography in monolayer cultures of hepatocytes, incubated in the presence of [methyl-3H] thymidine. Dimethyl disulphide did not induce DNA-repair activities in hepatocytes, either during the 4 h exposure period or during the subsequent 16 h or 24 h after the exposure period.

10.8.2 Comparison with the CLP criteria

Three bacterial assays for gene mutation with a negative result (Barfknecht, 1985; Jones, 1985 and Wagner, 2007), a test for clastogenicity in mammalian cells with a negative result at non-toxic doses (de Vogel, 1990), and a test for gene mutation in mammalian cells with no conclusive evidence of mutations (Rutten, 1990) are all available for dimethyl disulphide. All are GLP- and recognised test guideline-compliant. The perception of equivocal results in the latter two tests could conceivably have triggered the requirement to conduct the further GLP- and recognised test guideline-compliant in vitro DNA repair test with a negative result (Bichet, 1990), in vivo- in vitro DNA repair test with a negative result (Rutten, 1990), in vivo mouse

inhalation micronucleus test with a negative result (Willems, 1989), and in vivo rat inhalation micronucleus test with a negative result (Weinberg, 2007). In conclusion, DMDS did not have the intrinsic ability to induce mutations in germ cells

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Not classified according to REGULATION (EC) No 1272-2008.

RAC evaluation of germ cell mutagenicity
Summary of the Dossier Submitter's proposal Based on negative or equivocal results in several *in vitro* and *in vivo* studies DS concluded that DMDS does not require classification for germ cell mutagenicity. ***In vitro studies*** In a key study performed in accordance with OECD TG 471, DMDS was tested in the Bacterial Reverse Mutation Assay using Salmonella typhimurium tester strains TA98, TA100, TA1535 and TA1537 and Escherichia coli tester strain WP2uvrA in the presence and absence of Aroclor-induced rat liver S9 (Wagner, 2007). The assay was performed using the plate incorporation method. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate in the initial toxicity-mutation assay and 50, 150, 500, 1500 and 5000 µg per plate in the confirmatory mutagenicity assay. No positive mutagenic response was observed. Neither precipitate nor appreciable toxicity was observed. DMDS was concluded to be negative in the Bacterial Reverse Mutation Assay. In two other supporting OECD TG 471 bacterial reverse mutation tests, DMDS was negative in Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100, in the presence and absence of metabolic activation (Jones, 1985; Barfknecht, 1985). In a chromosome aberration study with human lymphocytes (De Vogel, 1990), a key study performed in accordance with OECD TG 473, DMDS did not induce a statistically significant increase in the number of cells with structural chromosome aberrations at non-toxic concentrations (≤ 100 µg/mL), both in the absence and in the presence of metabolic activation. At the clearly toxic concentration of 300 µg/mL, both in the absence and in the presence of metabolic activation, DMDS induced a statistically significant increase in the number of cells with structural chromosome aberrations. In a mammalian cell gene mutation assay (HGPRT) with CHO cells (Rutten, 1990), a key study performed in accordance with OECD TG 476, DMDS (0.46, 1.37, 4.12, 12.3, 37.0, 74.0, 111, 333, 667 and 1000 µg/mL) did not increase the mutant frequency in the absence of metabolic activation. In the presence of a metabolic activation system, DMDS induced a slight increase in mutant frequency at several concentrations. These increases in mutant frequency were not concentration-related or clearly reproducible. DMDS was highly toxic to CHO cells at a concentration range of 74 – 1 000 µg/mL. The actual concentrations of DMDS in culture medium were much lower than the target concentrations. Recovery experiments showed that during incubation, about 50% was lost directly presumably by evaporation and an additional 25% was lost presumably by reactions with constituents of the incubation. There was no conclusive evidence for a genotoxic effect of DMDS in cultured CHO cells.

In a DNA damage and repair assay (Bichet, 1990), a key study performed in accordance with OECD TG 482, DMDS (1, 5, 10, 50, 100, 200 and 300 µg/mL; cytotoxic > 100 µg/mL) was not genotoxic to rat hepatocytes in culture.

In vivo studies

In a key micronucleus assay performed following the OECD TG 474 and the OPPTS Guideline No. 870.5395, DMDS at concentrations of 217, 421 and 825 ppm did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow when male and female Sprague-Dawley rats were exposed to the test substance via a single, 4-hour, whole-body inhalation exposure (Weinberg, 2007).

In a supporting OECD TG 474 study, three groups of mice were exposed to atmospheres containing 0, 250 and 500 ppm DMDS for 6 hours a day for 4 consecutive days (Willems, 1989). Bone marrow cells were collected from the femur and examined for the presence of micronucleated poly- and normochromatic erythrocytes. Exposure to DMDS resulted in clear signs of toxicity at 250 ppm and 500 ppm, and 12/20 mice of the 500 ppm group died. Mean numbers of polychromatic erythrocytes were slightly lower in mice exposed to 500 ppm DMDS, suggesting slight cytotoxic effects on bone marrow cells. There were no increases in the incidences of micronucleated erythrocytes attributable to DMDS exposure.

In an unscheduled DNA synthesis test, a key study performed in accordance with OECD TG 482 (Rutten, 1990), male rats were exposed by inhalation to 500 ppm DMDS (maximally tolerated concentration) for 4 h. Immediately after the exposure and after the subsequent non-exposure periods of 16 and 24 h, animals were sacrificed for the isolation of hepatocytes. The DNA-repair activities were examined by autoradiography in monolayer cultures of hepatocytes, incubated in the presence of [methyl-^{3}H] thymidine. DMDS did not induce DNA-repair activities in hepatocytes, either during the 4 h exposure period or during the subsequent 16 or 24 h.

In a recent micronucleus assay (Randazzo, 2017) performed in accordance with the OECD TG 474 and GLP (a robust study summary was submitted to ECHA by the Dossier Submitter during the public consultation), DMDS whole body exposure at concentrations of 175, 350 and 700 ppm for 6 hours per day for 3 consecutive days did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow of male and female Sprague-Dawley rats. The percentage of micronucleated polychromatic erythrocytes was significantly increased in the positive control group (cyclophosphamide).

Comments received during public consultation

One MSCA agreed with the proposal for no classification for germ cell mutagenicity noting that based on the presented summaries, the conclusion "conclusive but not sufficient for classification" was supported.

Another MSCA considered that no conclusion could be drawn on the genotoxic potential of DMDS, without the results of the ongoing combined *in vivo* micronucleus assay and *in vivo* alkaline comet assay on DMDS. As an *in vivo* study, the study was also expected to reveal the effects of potential *in vivo* metabolites, noting that the major degradation products of DMDS in air via photo-oxidation were formaldehyde (25%), sulphur dioxide

(47%) and methanesulfonic acid (28%). The MSCA further noted that several of the currently available genotoxicity studies showed limitations and/or positive/equivocal results

In response, the DS indicated that at the time of PC, the micronucleus assay was already available and DMDS was negative at the maximal tolerated concentration of 700 ppm. The RSS of this study (Randazzo, 2017) was displayed in Appendix 1. The comet assay in nasal, liver and lung tissues had been just completed*. DMDS was negative in the liver and lung at the maximal tolerated concentration of 700 ppm and in nasal tissue at the maximal non cytotoxic concentration of 175 ppm. Furthermore, in the opinion of the DS, formaldehyde was not relevant for the toxicological evaluation of DMDS as none of the data available on DMDS and similar products indicated their metabolism in animals to aldehyde. The DS did not either agree with the MSCA regarding the interpretation that certain studies in the CLH report showed limitations or positive results. All in all, the DS considered that the data was conclusive but not sufficient for classification.

*ECHA note: The RSS of the comet assay was provided for RAC assessment by the DS after the public consultation.

Assessment and comparison with the classification criteria

Taking into account the analysis presented by the DS in the RCOM document and considering the negative results in all *in vitro* and *in vivo* tests (including also the new combined *in vivo* micronucleus assay and *in vivo* alkaline comet assay of DMDS) at doses/concentrations selected in line with OECD TG recommendations, RAC is of the opinion that DMDS **does not warrant classification for germ cell mutagenicity**.

10.9 Carcinogenicity

No data are available for that specific hazard class

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 20: Summary table of animal studies on adverse effects on sexual function and fertility

Method	Results	Remarks	Reference
rat (Sprague-Dawley) male/female two-generation study inhalation: vapour (whole body) 5, 20 and 80 ppm (19, 77 and 308 mg/m^3) (analytical conc.)	NOAEC (reproductive and developmental toxicity) (F0 and F1): 80 ppm (analytical) (male/female) (No functional effects on reproduction (estrous cycles, mating and fertility indices, number of days between pairing and coitus, and gestation length) in any	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Nemec MD (2006b)

Method	Results	Remarks	Reference
Vehicle: unchanged (no vehicle) Exposure: The F0 and F1 males and females were exposed to the test atmosphere for a minimum of 70 consecutive days prior to mating. Exposure of the F0 and F1 males continued throughout mating and through the day prior to euthanasia. The F0 and F1 females continued to be exposed throughout mating and gestation through gestation day 20. To prevent confounding effects on nursing, exposure for F0 and F1 females was suspended from gestation day 21 through lactation day 4, inclusively, and was re-initiated on lactation day 5 and continued through the day prior to euthanasia. During lactation (except when indicated above), the dams were removed from their litters during each daily 6-hour exposure period. (6 hours per day, 7 days per week) OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)	DMDS-exposed group. There were no adverse effects on pups born to dams exposed to DMDS and results from several studies confirm a lack of effect on postnatal growth prior to weaning with exposure of the lactating dams.) NOAEC (parental systemic toxicity) (F0 and F1): 5 ppm (analytical) (male/female) (persistent decrements in mean body weights, body weight gains and/or food consumption, increase in the incidence of vacuolization of the adrenal cortex or increased adrenal gland weights)		
rat (Sprague-Dawley) male/female screening inhalation: vapour (whole body) 5, 50 and 150 ppm (19, 192 and 577 mg/m^3) (analytical conc.) Vehicle: unchanged (no vehicle) Exposure: The F0 males and females were exposed to the test atmosphere for 14 consecutive days prior to mating. Exposure of the F0 males continued throughout mating and through the day prior to euthanasia. The F0 females continued to be exposed throughout mating through gestation day 20; exposure was re-initiated on	NOAEC (parental systemic toxicity) (P): 5 ppm (analytical) (male/female) (Decrements in body weight gain and food consumption in the 50 (males only) and 150 ppm groups.) NOAEC (parental reproductive toxicity) (P): 150 ppm (analytical) (male/female) (No functional effects on reproduction at any exposure concentration.) NOAEC (neonatal toxicity) (F1): 5 ppm (analytical) (male/female) (Reduced F1 pup body weights and body weight gains.)	1 (reliable without restriction) supporting study experimental result **Test material (EC name): dimethyl disulphide**	Nemec MD (2006c)

Method	Results	Remarks	Reference
lactation day 5 and continued through lactation day 27. During lactation, the dams were removed from their litters during each daily 6-hour exposure period. One F1 pup/sex/litter was selected for inhalation exposure beginning on PND 28 (following weaning) and continuing until PND 34. (6 hours per day, 7 days per week) OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test)			

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

A 2-generation reproduction study in rats was conducted to evaluate the potential adverse effects of dimethyl disulphide (DMDS) on male and female reproductive capabilities, including gonadal function, oestrous cyclicity, mating behaviour, conception, gestation, parturition, lactation and weaning of the F0 and F1 generations and F1 and F2 neonatal survival, growth and development (Nemec, 2006b). One litter per dam was produced in each generation. There were no functional effects on reproduction (oestrous cycles, mating and fertility indices, number of days between pairing and coitus, and gestation length) at any DMDS-exposed groups. There were no adverse effects on pups born to dams exposed to DMDS and results from several studies confirm a lack of effect on postnatal growth prior to weaning with exposure of the lactating dams. Therefore, in this study an exposure level of 80 ppm was considered to be the NOAEC for reproductive and developmental toxicity when DMDS was administered via whole-body inhalation to Sprague-Dawley rats. General systemic toxicity was evident in the 20 and 80 ppm group F0 and F1 parental males and females with persistent decrements in mean body weights, body weight gains and/or food consumption. Potential exposure-related effects on the adrenal glands (an increase in the incidence of vacuolization of the adrenal cortex or increased adrenal gland weights [relative to final body weight and brain weight]) were noted in the F0 and F1 parental animals in the 80 ppm group. At 5 ppm, The F0 and F1 parental groups were not affected showed slight transitory decreases in body weight gain and/or food consumption that were not considered adverse. Therefore, the NOAEC for parental toxicity was considered to be 5 ppm.

In a Reproduction / Developmental Toxicity Screening Test performed according to the OECD Guideline #421, 4 groups of male and female Crl: CD-SD) rats (12/sex/group) were exposed to either clean filtered air or vapor atmospheres of dimethyl disulphide (DMDS), for 6 hours daily for 14 consecutive days prior to mating (Nemec, 2006c). Target and measured test article concentrations were 0, 5, 50 and 150 parts per million (ppm). Exposure of the F0 males continued during the mating period and through the day prior to euthanasia for a total of 29 days of exposure. The F0 females continued to be exposed throughout mating and gestation through gestation day 20. After parturition, exposure of the F0 females was re-initiated on lactation day 5 and continued through the day prior to euthanasia. There were no functional effects on reproduction (mating and fertility indices, number of days between pairing and coitus, and gestation length) at any exposure concentration. Therefore, an exposure level of 150 ppm was considered to be the NOAEL (no-observed-adverse-effect level) for parental reproductive toxicity of DMDS when administered via whole-body inhalation exposure to rats. In general, evidence of general toxicity was more pronounced in the F0 males than in the F0 females and consisted of decrements in body weight gain and food consumption in the 50 (males only) and 150 ppm groups. Therefore, the NOAEL for parental systemic toxicity was considered to be 5 ppm. Neonatal toxicity was expressed at 50 and 150 ppm by reduced F1 pup body weights and body

weight gains. Mean F1 male and female body weights and body weight gains were reduced further after 1 week of direct test article exposure during the postweaning period. Therefore, the NOAEL for neonatal toxicity was 5 ppm.

10.10.3 Comparison with the CLP criteria

On the basis of the lack of reproductive toxicity in a 2-generation reproductive toxicity study in rat (Nemec, 2006b), dimethyl disulphide does not warrant classification for reproductive toxicity according to Regulation (EC) No 1272-2008.

10.10.4 Adverse effects on development

Table 21: Summary table of animal studies on adverse effects on development

Method	Results	Remarks	Reference
rat (Sprague-Dawley) inhalation: vapour (whole body) 5, 20 and 80 ppm (19, 77 and 308 mg/m^3) (analytical conc.) Vehicle: unchanged (no vehicle) Exposure: gestation days 6-19 (6 hours per day) OECD Guideline 414 (Prenatal Developmental Toxicity Study) EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study)	NOAEC (maternal toxicity): 20 ppm (analytical) (lower mean maternal body weight gains and food consumption at 80 ppm,) NOAEC (developmental toxicity): 20 ppm (analytical) (lower mean fetal weight and increased mean litter proportions of several skeletal variations at 80 ppm,) NOAEC (teratogenicity): >= 80 ppm (analytical) (No malformation observed up to 80 ppm)	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Nemec MD (2006d)
rabbit (New Zealand White) inhalation: vapour (whole body) 15, 45, 135 ppm (58, 173 and 519 mg/m^3) (analytical conc.) Vehicle: unchanged (no vehicle) Exposure: Gestation days 6-28 (23 exposures) (6 hours per day, 7 days per week) OECD Guideline 414 (Prenatal Developmental Toxicity Study) EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study)	NOAEC (maternal toxicity): >= 135 ppm (analytical) (Lack of adverse effects on maternal body weight gain, food consumption and survival) NOAEC (developmental toxicity): >= 135 ppm (analytical) (No developmental toxicity was observed up to 135 ppm) NOAEC (teratogenicity): >= 135 ppm (analytical) (No teratogenic effect was observed up to 135 ppm)	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Nemec MD (2005a)
rat (Sprague-Dawley)	NOAEC (maternal toxicity): 5 ppm	1 (reliable without restriction)	Barker L (1991)

Method	Results	Remarks	Reference
inhalation (whole body) 5, 15, 50 ppm (19, 58 and 192 mg/m^3) (analytical conc.) Vehicle: unchanged (no vehicle) Exposure: day 6 to day 15 of gestation (6 h/day) OECD Guideline 414 (Prenatal Developmental Toxicity Study)	(analytical) (Dose-related reductions in maternal weight gain at 15 and 50 ppm) NOAEC (teratogenicity): > 50 ppm (analytical) (No malformation observed up to 50 ppm) NOAEC (embryotoxicity/foetotoxicity): 15 ppm (analytical) (Reduced litter and foetal weights at 50 ppm)	supporting study experimental result **Test material (EC name): dimethyl disulphide**	

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

In a key developmental toxicity study performed following the OECD guideline # 414, four groups of 27 bred femaleCrl: CD(SD) rats were exposed to either filtered or vapor atmospheres of dimethyl disulphide (DMDS) for 6 hours daily in whole-body inhalation chambers during gestation days 6 through 19 (Nemec, 2006d). Test concentrations were 0, 5, 20 and 80 ppm (0, 19, 77 and 308 mg/m^3). All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and food consumption were recorded at appropriate intervals. On gestation day 20, a laparohysterectomy was performed on each female. The uterus, placenta and ovaries were examined and the number of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed and examined for external, visceral and skeletal malformations and developmental variations. The maternal LOAEL was 80 ppm based on lower mean maternal body weight gains and food consumption. The NOAEL for maternal toxicity was 20 ppm. The fetal/developmental toxicity LOAEL was also 80 ppm based on lower mean fetal weight and increased mean litter proportions of several skeletal variations. The NOAEL for fetal/developmental toxicity was 20 ppm, no teratogenic effect was observed.

In an earlier OECD 414 study, three groups of 30 mated female rats were exposed to DMDS by whole body exposure at 0, 5, 15 or 50 ppm (0, 19, 58 and 192 mg/m^3) for 6 hours daily from day 6 to 15 of gestation (Barker, 1991). All animals were killed on day 20 of gestation, and their uterine contents assessed. There were no deaths. A higher incidence of rough hair coat was observed at 50 ppm. Reductions in weight gain were observed at 15 and 50 ppm and food intake was reduced at 50 ppm. Litter and fetal weights were reduced at 50 ppm. No malformations were observed in fetuses from the treated groups. A slightly higher incidence of retarded ossification was observed at 50 ppm. Exposure to DMDS at 50 ppm elicited maternal toxicity with associated fetal growth retardation, which was demonstrated by low weight and retarded ossification. The NOAEL was 5 ppm for maternal toxicity, and 15 ppm for embryofetal effects (Barker, 1991).

Dimethyl disulphide (DMDS) was evaluated in an inhalation prenatal developmental toxicity study in rabbits performed following the OECD guidleine # 414 (Nemec, 2005a). DMDS was administered via whole-body inhalation as a vapor to three groups of 24 time-mated female New Zeeland White rabbits on a 6-hoursper day basis during gestation days 6 through 28. A concurrent control group of 24 time-mated rabbits were exposed to clean, filtered air on a comparable regimen. The DMDS exposure concentrations were 0, 15, 45 and 135 ppm (0, 58, 173 and 519 mg/m^3). Transient clinical observations were noted at 45 and 135 ppm on the first day of exposure only and decreased food consumption at 135 ppm was sustained throughout the first 2 weeks of exposure but in the absence of effects on maternal body weight gains these findings were not

considered adverse. Food consumption in the 15 and 45 ppm groups and mean body weights, body weight gains, net body weights, net body weight gains and gravid uterine weights in the 15, 45 and 135 ppm groups were unaffected by DMDS exposure for the duration of the study. A macroscopic finding of dark red discoloration of or dark red areas on the lungs (generally all lobes) was noted at all exposure levels. Intrauterine growth and survival was unaffected by test article exposure and no test article related malformations or developmental variations were noted at any exposure level. An exposure level of 135 ppm was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity (based on the lack of adverse effects on maternal body weight gain, food consumption and survival) and an exposure level of 135 ppm was considered to be the NOAEL for developmental toxicity when dimethyl disulphide was administered via whole-body inhalation to rabbits.

10.10.6 Comparison with the CLP criteria

Considering the lack of teratogenic effects and of developmental toxicity at concentrations lower than the concentrations inducing maternal toxicity in developmental toxicity studies in rat and rabbit, dimethyl disulphide does not warrant classification for developmental toxicity according to Regulation (EC) No 1272-2008.

10.10.7 Adverse effects on or via lactation

No adverse effect was observed during the 2-generation study.

10.10.8 Conclusion on classification and labelling for reproductive toxicity

No classification is warranted.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The following studies were provided for assessment of reproductive toxicity:

- A two-generation study via inhalation in rats (Nemec, 2006b)
- A reproduction/developmental toxicity screening test (Nemec, 2006c)
- A prenatal developmental toxicity study in rats (Nemec, 2006d)
- A prenatal developmental toxicity study in rabbits (Nemec, 2005a)
- A prenatal developmental toxicity study in rats (Barker, 1991)

In Annex I, there were two additional studies that were not assessed by the DS; a lactational inhalation phased-exposure study of DMDS in rats (Nemec, 2006) and an inhalation range-finding study on DMDS in the pregnant rat (Barker, 1991).

Sexual function and fertility

The adverse effects of DMDS on sexual function and fertility were assessed based on results of the two-generation study on rats exposed by inhalation to DMDS vapour performed according to OECD TG 416 (Nemec, 2006b) and on results of the reproduction/developmental toxicity screening inhalation study of DMDS in rats according to OECD TG 421 (Nemec MD, 2006c).

In the two-generation study via inhalation in rats (Nemec, 2006b), the F0 and F1 males and females were exposed to DMDS vapour (6 hours per day, 7 days per week) at concentrations of 0, 5, 20 and 80 ppm (0, 19, 77 and 308 mg/m^3) for a minimum of 70 consecutive days prior to mating. Exposure of the F0 and F1 males continued throughout mating and until the day prior to euthanasia. The F0 and F1 females continued to be exposed throughout mating and gestation until gestation day 20. To prevent confounding effects on nursing, exposure for F0 and F1 females was suspended from gestation day 21 through lactation day 4, inclusive, and was re-initiated on lactation day 5 and continued until the day prior to euthanasia. During lactation (except when indicated above), the dams were removed from their litters during each daily 6-hour exposure period.

No adverse effects on sexual function and fertility (estrous cycles, mating and fertility indices, number of days between pairing and coitus, and gestation length) were observed in any DMDS-exposed group. No parental systemic toxicity (F0 and F1) was noted only at 5 ppm (19 mg/m3) (male/female) while at higher concentrations of 20 and 80 ppm (77 and 308 mg/m^3) a persistent decrease in mean body weights, body weight gains and/or food consumption, increase in the incidence of vacuolisation of the adrenal cortex or increased adrenal gland weights were observed.

In the reproduction/developmental toxicity screening test (Nemec, 2006c), 4 groups of male and female Crl: CD-SD) rats (12/sex/group) were exposed to either clean filtered air or vapour atmospheres of DMDS, for 6 hours daily for 14 consecutive days prior to mating (Nemec, 2006c). Target and measured test substance concentrations were 0, 5, 50 and 150 ppm (0, 19, 192 and 577 mg/m^3). Exposure of the F0 males continued during the mating period and through the day prior to euthanasia for a total of 29 days. The F0 females continued to be exposed throughout the mating and gestation until gestation day 20. After parturition, exposure of the F0 females was re-initiated on lactation day 5 and continued until the day prior to euthanasia. The DS concluded that there were no adverse effects on sexual function and fertility (mating and fertility indices, number of days between pairing and coitus, and gestation length) at any exposure concentration. In general, evidence of parental toxicity was more pronounced in the F0 males than in the F0 females and consisted of decrease in body weight gain and food consumption in the 50 (males only) and 150 ppm groups.

Based on the above data DS did not propose classification of DMDS for effects on sexual function and fertility.

Developmental toxicity

In addition to the studies by Nemec (2006b) and (2006c), there were three additional studies relevant for developmental toxicity; two developmental toxicity studies in rats (Barker, 1991, Nemec, 2006d) and one in rabbits (Nemec, 2005a).

Rats

In the two-generation study (Nemec, 2006b) on rats performed according to the OECD TG 416**,** there were no adverse effects on pups born to dams exposed to DMDS and according to the DS the results confirmed the lack of effect on postnatal growth prior to weaning with exposure of the lactating dams. Developmental toxicity was not observed up to a concentration of 80 ppm (308 mg/m3).

In the reproduction/developmental toxicity screening test (Nemec, 2006c) mean F1 male and female pup body weight gains were lower in the 50 and 150 ppm groups during PND 4-28 (females) and PND 7-14 (males), when the dams were again exposed to DMDS. As a result,

mean body weights of pups in the 50 and 150 ppm groups were up to 15.3% and 17.0% less (females) and up to 11.2% and 6.7% less (males) than in the control group, respectively. According to the DS, these decreases in body weights of pups might have been related to decreases in body weight gain and food consumption of parental animals in the 50 (males only) and 150 ppm groups (males and females).

In an OECD TG 414 study (Barker, 1991), three groups of 30 mated female rats were exposed to DMDS by whole body inhalation exposure at 0, 5, 15 or 50 ppm (0, 19, 58 and 192 mg/m^3) for 6 hours daily from day 6 to 15 of gestation. All animals were killed on day 20 of gestation, and their uterine contents assessed.

Maternal toxicity

There were no deaths. A higher incidence of rough hair coat was observed at 50 ppm. Dose-related reductions in weight gain were observed at 15 and 50 ppm (58 and 192 mg/m^3). At 50 ppm (192 mg/m^3), weight gain was 40% lower than in controls over the exposure period (day 6 to 15, $p < 0.001$). At 15 ppm (58 mg/m^3), weight gain over the exposure period was 16% lower than in the control group on days 6 to 15, $p< 0.01$). Food intake was lower ($p< 0.001$) than in controls at 50 ppm (192 mg/m^3), but comparable to controls at 5 or 15 ppm (19 and 58 mg/m^3).

Developmental effects

Litter and foetal weights were slightly but significantly reduced at 50 ppm.

Summary of number of foetuses and litter weights (g):

	0 ppm	5 ppm	15 ppm	50 ppm
Number of male foetuses	128	73	152	119
Number of female foetuses	130	89	143	115
%male foetuses	49.6	45.1	51.5	50.9
Mean litter weight	43.7	41.8	41.7	38.8
Mean foetal weight	3.8	3.9	3.7	3.5**
Mean foetal weight males only	4.0	4.1	3.8	3.6**
Mean foetal weight females only	3.7	3.7	3.6	3.4**

No malformations were observed in foetuses from the treated groups. A slightly higher incidence of retarded ossification was observed at 50 ppm.

Foetal defect data:

	0 ppm	5 ppm	15 ppm	50 ppm
EXTERNAL AND VISCERAL DEFECTS				
Number of foetuses examined	258	162	295	234
Number showing malformations	1	0	0	0
% of foetuses examined	0.4	0.0	0.0	0.0
Number showing variations	52	27	65	52
% of foetuses examined	20.2	16.7	22.0	22.2
SKELETAL DEFECTS				
Number of foetuses examined	136	85	155	121
Number showing malformations	1	0	0	0

% of foetuses examined	0.7	0.0	0.0	0.0
Number showing variations	123	74	139	116
% of foetuses examined	90.4	87.1	89.7	95.9
Total number of foetuses showing malformations	1	0	0	0
% of foetuses examined	0.4	0.0	0.0	0.0

Exposure to DMDS at 50 ppm elicited maternal toxicity with associated foetal growth retardation, which was demonstrated by low weight and retarded ossification. The NOAEL was 5 ppm for maternal toxicity, and 15 ppm for embryofoetal effects (Barker, 1991).

In a key developmental toxicity study (Nemec, 2006d) performed in accordance with the OECD TG 414, four groups of 27 bred female Crl: CD(SD) rats were exposed to either filtered or vapour atmospheres of DMDS for 6 hours daily in whole-body inhalation chambers during gestation days 6 through 19 (Nemec, 2006d). Test concentrations were 0, 5, 20 and 80 ppm (0, 19, 77 and 308 mg/m^3). All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and food consumption were recorded at appropriate intervals. On gestation day 20, a laparohysterectomy was performed on each female. The uterus, placenta and ovaries were examined and the number of foetuses, early and late resorptions, total implantations and number of corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The foetuses were weighed, sexed and examined for external, visceral and skeletal malformations and developmental variations.

Maternal toxicity

The maternal LOAEL was 80 ppm (308 mg/m^3) based on lower mean maternal body weight gains and food consumption. The NOAEL for maternal toxicity was 20 ppm (77 mg/m^3).

Developmental effects

Mean fetal weight in the 80 ppm (308 mg/m^3) group (3.0 g) was lower than the concurrent control group value (3.7 g) and the minimum mean value in the historical control data (3.4 g). The difference from the concurrent control group was statistically significant ($p<0.01$) and was considered test substance-related. Post-implantation loss, live litter size and fetal sex ratios in the 80 ppm (308 mg/m^3) group were unaffected by maternal test article exposure. Intrauterine growth and survival were unaffected by the test article at exposure levels of 5 and 20 ppm. Mean numbers of corpora lutea and implantation sites and the mean litter proportions of preimplantation loss were similar across all groups.

The numbers of fetuses (litters) available for morphological evaluation were 405(27), 405(26), 406(27) and 408(26) in the control, 5, 20 and 80 ppm groups, respectively. Malformations were observed in 2(2), 1(1), 2(2) and 2(1) fetuses (litters) in these same respective exposure groups.

External malformations were noted for 1, 0, 1 and 2 fetuses in the control, 5, 20 and 80 ppm groups, respectively, and included the following: microphthalmia (left orbit appeared smaller than normal) in fetus no. 31383-03 in the control group and fetus nos. 31480-13 and 31480-18 in the 80 ppm group. Fetus no. 31480-13 in the 80 ppm group also had anal atresia and vertebral agenesis (all vertebrae posterior to lumbar vertebra no. 4 absent). The only other external malformation observed in this study, localized fetal edema (neck and thorax), was noted for fetus no. 31402-17 in the 20 ppm group. Because these external malformations were observed in single fetuses, they were also observed in the control group, and/or they were

observed in a manner that was not related to maternal exposure concentration, none were considered test substance-related by the DS. No external developmental variations were noted for fetuses in this study.

Visceral malformations consisted of hydrocephaly (increased cavitation of both lateral ventricles and the third ventricle) in control group fetus no. 31467-06, and a malpositioned oesophagus (located to the right of the trachea) and lobular dysgenesis of the lungs (all right lobes were fused) in fetus no. 31478-03 in the 5 ppm group. Because no soft tissue malformations were noted in the 20 and 80 ppm groups, the soft tissue malformations noted at 5 ppm were not considered test article-related. Visceral developmental variations noted in the 5 and 20 ppm groups consisted of renal papillae not developed (Woo and Hoar grade 0) and/or distended ureters, and an accessory spleen. These variations were not considered to be test substance-related because there were no visceral developmental variations noted for fetuses in the 80 ppm group.

The only fetal skeletal malformation in this study, sternoschisis (sternal band nos. 1-6 not joined), was noted for a single fetus (no. 31444-04) in the 20 ppm group. Because no skeletal malformations were noted at 80 ppm, this malformation was not considered test substance-related. Test substance-related differences in the mean litter proportions of skeletal developmental variations were noted in the 80 ppm group. These differences included increased mean litter proportions of unossified sternebrae nos. 5 and/or 6, unossified sternebrae nos. 1, 2, 3 and/or 4, reduced ossification of the vertebral arches, unossified pubis and unossified hyoid, and a decreased mean litter proportion of ossified cervical centrum no. 1 at 80 ppm. Only the difference for unossified sternebrae nos. 5 and/or 6 was statistically significant ($p<0.01$) compared to the concurrent control group. These skeletal variations were considered test article-related because they corresponded to the reduced mean fetal body weight at 80 ppm, indicative of developmental delay, and were occasionally outside of the WIL historical control data range. The mean litter proportions of maligned sternebrae in the 5, 20 and 80 ppm groups (1.0%, 1.7% and 1.3% per litter, respectively) were higher than the concurrent control group value (0.3% per litter), but did not exceed the range of mean values in the WIL historical control data (0.0% to 1.7% per litter) and did not occur in a manner that was exposure-related. Therefore, maligned sternebrae in these groups were not considered test substance-related. Skeletal developmental variations noted in the 5 and 20 ppm groups consisted primarily of unossified sternebrae nos. 5 and/or 6, ossified cervical centrum no. 1, and 14th rudimentary ribs. These variations were not considered test substance-related because the mean litter proportions in these groups were similar to control group values.

The foetal/developmental toxicity LOAEL was also 80 ppm (308 mg/m^3) based on lower mean foetal weight and increased mean litter proportions of several skeletal variations. The NOAEL for foetal/developmental toxicity was 20 ppm (77 mg/m^3), no teratogenic effect was observed.

Rabbits

DMDS was evaluated in an inhalation prenatal developmental toxicity study in rabbits performed following the OECD TG 414 (Nemec, 2005a). DMDS was administered via whole-body inhalation as a vapour to three groups of 24 time-mated female New Zeeland White rabbits on a 6-hours per day basis during gestation days 6 through 28. A concurrent control group of 24 time-mated rabbits was exposed to clean, filtered air on a comparable regimen. The DMDS exposure concentrations were 0, 15, 45 and 135 ppm (0, 58, 173 and 519 mg/m3).

Maternal toxicity

Transient clinical observations were noted at 45 and 135 ppm on the first day of exposure only

and decreased food consumption at 135 ppm was sustained throughout the first 2 weeks of exposure but in the absence of effects on maternal body weight gains these findings were not considered adverse. Food consumption in the 15 and 45 ppm groups and mean body weights, body weight gains, net body weights, net body weight gains and gravid uterine weights in the 15, 45 and 135 ppm groups were unaffected by DMDS exposure. A macroscopic finding of dark red discoloration of or dark red areas on the lungs (generally all lobes) was noted at all exposure levels.

Developmental effects

Intrauterine growth and survival were unaffected by test substance administration at exposure levels of 15, 45 and 135 ppm. Parameters evaluated included post-implantation loss, live litter size, foetal body weights and foetal sex ratios. Mean numbers of corpora lutea and implantation sites and the mean litter proportions of preimplantation loss were similar across all groups.
There were no external malformations or developmental variations noted in the 15, 45 and 135 ppm groups. In the control group, two foetuses had umbilical herniation of the intestine (several loops of the intestine protruded through an opening in the umbilicus).

Soft tissue malformations were observed in 3(3), 2(1), 3(1) and 3(2) foetuses (litters) in the control, 15, 45 and 135 ppm groups, respectively.

Skeletal malformations were observed in 6(3), 3(3) and 2(2) foetuses (litters) in the control, 15 and 45 ppm groups, respectively. No skeletal malformations were noted at the 135 ppm exposure level; therefore, the skeletal malformations were not considered exposure-related. The percent per litter of 13th full ribs in the 45 and 135 ppm groups (44.8% and 55.5% per litter, respectively) were increased compared to the control group (31.3% per litter). Although the difference was statistically significant ($p<0.05$) for the 135 ppm group, the values were within the WIL historical control data range (19.4% - 59.1% per litter) and in the absence of other indicators of developmental toxicity was not considered related to exposure.

An exposure level of 135 ppm (519 mg/m3) was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity (based on the lack of adverse effects on maternal body weight gain, food consumption and survival) and an exposure level of 135 ppm (519 mg/m^3) was considered to be the NOAEL for developmental toxicity when DMDS was administered via whole-body inhalation to rabbits.

Based on the above data DS did not propose classification of DMDS for effects on development.

Adverse effects on or via lactation

A lactational inhalation phased-exposure study of DMDS in rats (Nemec, 2006) was conducted in order to determine if the pup body weight effects noted in the concurrent two-generation study (Nemec, 2006b) at exposure levels of 5, 20 and 80 ppm were a true reflection of toxicity, and was designed to examine whether more abbreviated exposure regimens targeted during lactation or more sustained exposure was necessary to replicate the effect on pup body weights. Based on the results of this study, no effects were noted on pup body weights when dams were exposed to DMDS at concentrations of 5, 20 and 80 ppm for 1 week during lactation days 5-12 (subset I) or lactation days 13-20 (subset II), or for 2 weeks during lactation days 5-20 (subset III). Therefore, the body weight effects noted for the F1 pups in

the concurrent two-generation study (WIL-160122) at 5, 20 and 80 ppm were not replicated in the current study (WIL-160126) when abbreviated exposure regimens were targeted.

DS did not propose classification for adverse effects on or via lactation

Comments received during public consultation

One MSCA agreed with a proposal of no classification of DMDS for reproductive toxicity noting that based on the presented summaries, the conclusion "conclusive but not sufficient for classification" was supported.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

1) A two-generation study via inhalation in rats (Nemec, 2006b)

F0 generation adverse effects on sexual function and fertility:

F0	0	5	20	80
Mating index (%)	100	100	100	93.3
Male copulation index (%)	96.7	93.3	96.7	90
Female conception index (%)	96.7	93.3	96.7	96.4
Males that did not sire a litter	1	2	1	3
Females that had evidence of mating but did not deliver	1	2	1	1
Mean gestation length (days)	22	21.8	21.9	21.9
Ovarian primordial follicle counts (mean)	60.9	NA	NA	61.9

The mean lengths of estrous cycles in the 5, 20 and 80 ppm groups were similar to the controls; 4.7, 4.4, 4.2 and 4.5 days at 0, 5, 20 and 80 ppm, respectively.
No exposure-related effects were observed in F0 spermatogenesis endpoints (mean testicular and epididymal sperm numbers and sperm production rate, motility, progressive motility and morphology) in males at any dosage concentration as the differences from the control group were slight and not statistically significant. At 80 ppm there was a statistically significant increase in the following organ weights (relative to body weight) as compared to controls; right cauda epididymis, left and right epididymis and left and right testis.

F0	0	5	20	80
Sperm motility (%),	79	81	83	83

mean				
Sperm progressive motility (%), mean	62	65	68	67
Sperm production rate (millions/gram/day)	11.3	11.3	11.2	11.1
% of morphologically normal sperm	99.4	99.7	98.3	99.3
Weight of right cauda epididymis (g / 100 g body weight)	0.062	0.061	0.062	0.069**
Weight of left cauda epididymis (g / 100 g body weight)	0.062	0.060	0.060	0.066
Weight of right epididymis (g / 100 g body weight)	0.137	0.132	0.136	0.151**
Weight of left epididymis (g / 100 g body weight)	0.130	0.128	0.126	0.141**
Weight of right testis (g / 100 g body weight)	0.321	0.310	0.323	0.372**
Weight of left testis (g / 100 g body weight)	0.320	0.309	0.317	0.364**

**Significantly different from the control group at 0.01 using Dunnett's test

F0 parental toxicity

No mortality was observed at any dose.

The mean body weight gain in the 80 ppm F0 male group (200 g) was statistically significantly ($p<0.01$) reduced during the entire pre-mating exposure period (study weeks 0-10) when compared to a mean body weight gain (266 g) in the control group. The cumulative mean body weight changes in the 20 ppm F0 male group during this same exposure interval was only slightly lower (not statistically significant) when compared to the control group and indicated a partial recovery during the first 2 weeks of exposure.

F0 males	0	5	20	80
BW gain (g) week 0-10	266	261	245	200**
Brain weight (g / 100 g body weight)	0.383	0.375	0.386	0.435**
Kidneys weight (g / 100 g body weight)	0.630	0.645	0.633	0.662
Liver weight (g / 100 g body weight)	3.110	3.159	3.231	3.178

Spleen weight (g / 100 g body weight)	0.142	0.143	0.139	0.141

**Significantly different from the control group at 0.01 using Dunnett's test

In F0 dams, the mean body weight gains in the control, 20 and 80 ppm groups were 154, 144 and 133 g, respectively, during the pre-mating period (study weeks 0-10), being statistically significantly (p<0.01) different between the control and 80 ppm groups.

F0 females	0	5	20	80
BW gain (g) week 0-10	154	151	144	133**
Brain weight (g / 100 g body weight)	0.592	0.594	0.603	0.628*
Adrenal glands weight (g / 100 g body weight)	0.021	0.022	0.021	0.024**

*Significantly different from the control group at 0.05 using Dunnett's test

**Significantly different from the control group at 0.01 using Dunnett's test

There were no exposure-related macroscopic changes noted at the scheduled necropsy.

There was an increased incidence of adrenocortical cytoplasmic vacuolisation in males at 80 ppm, but not at 5 and 20 ppm as compared to controls (6/30, 7/30, 6/30 and 12/30 at 0, 5, 20 and 80 ppm, respectively).

The relative brain weight was increased in males and females at 80 ppm compared to controls. Also in males at 80 ppm, there was a statistically significant decrease in the weight of kidney, liver and spleen (absolute and relative to brain weight) compared to controls. In females at 80 ppm, there was a statistically significant increase in the weight of adrenal glands (relative to body weight) compared to controls.

F1 generation adverse effects on sexual function and fertility:

F0	0	5	20	80
Mating index (%)	93.3	89.7	100	93.3
male and female fertility index (%)	83.3	86.2	93.1	90
Male copulation and female conception index (%)	89.3	96.2	93.1	96.4
Males that did not sire a litter	5	4	2	3
Females that had evidence of mating but	3	1	2	1

did not deliver				
Mean gestation length (days)	22.1	21.8	21.7	22.1
Ovarian primordial follicle counts (mean)	61.5	No data	No data	77.9
Mean length of estrous cycles	5.2	4.4	5.7	4.7
Implantation sites	13	14.8	14.7	13

The mean number of days between pairing and coitus and the mean length of estrous cycles in the test substance-exposed groups were similar to the control group values. Furthermore, no exposure-related effects were observed on F1 spermatogenesis endpoints (mean testicular and epididymal sperm numbers and sperm production rate, motility, progressive motility and morphology) at any dosage concentration. There were statistically significant changes in the absolute and/or relative weights of epididymis and testis at the top dose or at multiple doses as compared to controls.

F1	0	5	20	80
Sperm motility (%), mean	86	85	84	83
Sperm progressive motility (%), mean	72	70	69	68
Sperm production rate (millions/gram/day)	12.3	12.7	13.2	12.8
% of morphologically normal sperm	99.7	99.8	99.8	99.7
Weight of right cauda epididymis (g / 100 g body weight)	0.061	0.064	0.068*	0.069*
Weight of left cauda epididymis (g / 100 g body weight)	0.055	0.060	0.063*	0.061
Weight of right epididymis (g / 100 g body weight)	0.80	0.76	0.78	0.75**
Weight of left epididymis (g / 100 g body weight)	0.116	0.128	0.134**	0.134**
Weight of right testis (g / 100 g body weight)	0.309	0.304	0.429	0.340*

Weight of left testis (g / 100 g body weight)	0.305	0.302	0.327	0.338*

*Significantly different from the control group at 0.05 using Dunnett's test

**Significantly different from the control group at 0.01 using Dunnett's test

F1 parental toxicity

No test substance-related deaths occurred.

Body weight gain during the pre-mating period (study weeks 18-30): cumulative mean body weight changes in the 20 and 80 ppm male and 80 ppm female groups were statistically significantly ($p<0.01$) reduced during the pre-mating period when compared to the control group. At 5 ppm, cumulative mean body weight gain was similar to the control group value.

There were no exposure-related macroscopic changes noted at the scheduled necropsy.

A mammary adenocarcinoma was identified in one female at 20 ppm.

There were also statistically significant changes in the absolute and/or relative weights of liver, kidneys, spleen, adrenal glands and pituitary at the top dose or all doses compared to controls.

F1 males	0	5	20	80
BW gain (g) week 18-30	436	412	390**	366**
Brain weight (g / 100 g body weight)	0.358	0.366	0.392**	0.405**
Kidneys weight (g / 100 g body weight)	0.606	0.641	0.625	0.631
Adrenal glands weight (g / 100 g body weight)	0.009	0.010	0.010	0.011**
Spleen weight (g / 100 g body weight)	0.147	0.147	0.153	0.144
Pituitary weight (g / 100 g body weight)	0.003	0.003	0.003	0.003

F1 females	0	5	20	80
BW gain (g) week 18-30	210	225	210	184**
Brain weight (g / 100 g body weight)	0.582	0.572	0.599	0.647**
Adrenal glands weight (g / 100 g body weight)	0.020	0.021	0.021	0.025**

**Significantly different from the control group at 0.01 using Dunnett's test

Since there were no significant adverse effects of DMDS on length of oestrous cycle, fertility indexes, gestation length and spermatogenesis at concentration moderately toxic to parental animals, RAC concludes that in this study there are no DMDS-induced adverse effects on sexual function and fertility.

2) A reproduction/developmental toxicity screening test (Nemec, 2006c)

The mean number of implantation sites were 15.3, 16.4, 16.1 and 13.9 at 0, 5, 50 and 150 ppm, respectively. There were no effects on mating and fertility indices, number of days between pairing and coitus, and gestation length at any exposure concentration.

	0	5	50	150
Mating indices (%)	100	91.7	100	100
Fertility indices (%)	91.7	83.3	100	91.7
Male copulation and female conception indices (%)	91.7	90.9	100	91.7
Gestation length	21.9	22	21.9	22

As a summary, taking into account that no treatment-related, adverse effects on sexual function and fertility were reported in these relevant and acceptable studies, RAC is of the opinion that DMDS does not warrant classification for adverse effects on sexual function and fertility.

Adverse effects on development

1) A two-generation study via inhalation in rats (Nemec, 2006b)

F1 generation adverse effects on development

The mean number of pups born, live litter size, percentage of males per litter at birth and postnatal survival between birth and PND 0 (relative to number born), PND 0-1, 1-4 (pre-selection), 4 (post-selection)-7, 7-14, 14-21, and from birth to PND 4 (pre-selection) and PND 4 (post-selection)-28 were unaffected by the test substance at all exposure concentrations. Differences from the control group were slight, not statistically significant and not occurring in an exposure-related manner. The numbers of F1 pups found dead and/or missing, as well as the general physical condition of all F1 pups in this study were unaffected by parental test article exposure.

F1	0	5	20	80
The mean number	14.7	15.1	14.6	15.3

of pups born				
Live litter size	14.6	14.8	14.2	15.2
Percentage of males per litter at birth	52.6	54.9	52.5	51.8
Postnatal survival between birth and PND 0	99	98	97.6	99.7
Postnatal survival PND 0-1	99.2	98.9	98.4	97.1
Postnatal survival PND 1-4	99.3	98.3	91.5	98.5
Postnatal survival PND 4-7	98.9	99.1	100	99.1
Postnatal survival PND 7-14	99.6	100	100	99.1
Postnatal survival PND 14-21	99.6	100	100	100
Postnatal survival from birth to PND 4	97.6	95.2	89.1	95.5
Postnatal survival PND 4-28	97.6	99.1	100	98.3

The numbers of pups (litters) found dead or euthanised *in extremis* during PND 0-28 were as follows; 12(11), 16(12), 40(11) and 14(8) in the control, 5, 20 and 80 ppm groups, respectively.

Mean ages of attainment of balanopreputial separation were 47.7, 45.8 and 46.7 days in the 5, 20 and 80 ppm groups, respectively, compared to 44.9 days in the concurrent control group; the difference from control was statistically significant in the 5 ppm group. The mean value for the age of attainment of balanopreputial separation age in the WIL historical control data for inhalation studies was 46.3 days.

Mean age at attainment of vaginal patency in the 5, 20 and 80 ppm groups was 36.2, 35.9 and 36.4 days, respectively, compared to 34.4 days in the concurrent control group. The differences in these same respective groups were statistically significant ($p<0.05$ or $p<0.01$) when compared to the concurrent control group. The mean value in the WIL historical control data for inhalation studies was 35.2 days with a range of 32.5 to 38.8 days.

Prolongation of mean ages of attainment of balanopreputial separation in male offspring and mean age at attainment of vaginal patency in the 5, 20 and 80 ppm groups did not depend on the level of exposure and the observed values were within a range of historical control values, thus they do not provide sufficient evidence of effect of DMDS on postnatal development.

F1 - pre-weaning - summary of offspring weights [g] (litter as experimental unit):

GROUP:	SEX		0 PPM	5 PPM	20 PPM	80 PPM
PND 1	MALES	MEAN	7.2	7.1	7.1	7.1
		S.D.	0.73	0.71	0.78	0.60
		N	29	28	29	27
	FEMALES	MEAN	6.7	6.7	6.7	6.7
		S.D.	0.58	0.76	0.66	0.54
		N	29	28	29	27
PND 4 (BEFORE SELECTION)	MALES	MEAN	10.1	9.8	10.2	10.1
		S.D.	1.47	1.37	1.27	1.11
		N	29	28	28	27
	FEMALES	MEAN	9.3	9.4	9.6	9.6
		S.D.	1.26	1.42	1.13	1.05
		N	29	28	28	27
PND 7	MALES	MEAN	13.8	12.7	13.3	13.5
		S.D.	2.28	1.80	1.96	1.97
		N	29	28	28	27
	FEMALES	MEAN	12.8	12.1	12.6	12.7
		S.D.	2.18	2.02	1.82	1.87
		N	29	28	28	27
PND 14	MALES	MEAN	26.4	23.8*	24.5	24.7
		S.D.	3.69	3.71	3.65	2.75
		N	29	28	28	27
	FEMALES	MEAN	24.9	22.9	23.5	23.4
		S.D.	3.92	3.94	3.49	2.48
		N	29	28	28	27
PND 21	MALES	MEAN	42.0	36.4**	38.2*	37.9
		S.D.	6.47	6.66	6.30	5.79
		N	29	28	28	27
	FEMALES	MEAN	39.6	35.1*	36.7	36.2
		S.D.	6.10	6.50	6.15	5.28
		N	29	28	28	27
PND 28	MALES	MEAN	79.7	70.1**	72.8*	72.0*
		S.D.	10.	66 11.	70 10.	94 9.88
		N	29	28	28	27
	FEMALES	MEAN	72.7	65.6*	67.4	66.1*
		S.D.	9.99	10.58	9.	67 8.36
		N	29	28	28	27

The mean F1 offspring weight was similar in control and experimental groups until PND 7. On postnatal day 14 the male offspring weight at 5 ppm, but not at 20 and 80 ppm, was lower than in the control group. The weight of female offspring was not affected by PND 14. On postnatal day 21 the male offspring weight in the 5 and 20 ppm group, but not in the 80 ppm group, was lower than in the control group. The female offspring weight was lower on PND 21 only in the 5 ppm group, but not in the 20 and 80 ppm groups. Only on PND 28 was the offspring weight lower than in the control in all treated groups (except in females in 20 ppm group). However, the weight in the 80 ppm group was less than 10% lower than in the control pups and no dose-response relationship was observed.

Comparison of the F1 pup data with the historical control values was confounded by the fact that the PND 4 pups in the test substance-exposed groups had mean body weights slightly above the mean values in the WIL historical control data for inhalation studies, while the PND 28 values in these groups were slightly lower (7% or less) than the mean values in the historical control data. In addition, mean body weights in the concurrent control group were notably higher (4.2% to 5.3%) when compared to the mean values in the WIL historical control data for inhalation studies on PND 28.

Since no dose-dependent reduction in body weight was seen in the F1 offspring up to PND 14, when pups are totally dependent on the milk of their mothers, RAC considers that there is not sufficient evidence of the effect of DMDS on or via lactation. The slight, not dose-dependent reduction in offspring weight might be due to biological variability or secondary non-specific

consequence of maternal toxicity. Slightly lower mean body weights (4.8% to 7.4%) in the 80 ppm F0 female group were noted during lactation days 1-28 compared to the mean body weights in the control group; the differences from control values were statistically significant ($p<0.05$ or $p<0.01$). No exposure-related effects were noted in mean body weights in the 5 and 20 ppm groups or in mean body weight changes in 5, 20 and 80 ppm during the lactation period when compared to the control group values.

F0 maternal toxicity:

F0 maternal body weight change during gestation (g)*	0	5	20	80
GD 0-4	19	17	19	15
GD 4-7	11	9	8	10
GD 7-11	17	19	18	17
GD 11-14	13	13	13	13
GD 14-17	28	29	30	29
GD 17-20	46	46	46	41
GD 0-20	133	133	135	125

* nongravid weight(s) not included in calculation of mean

F0 maternal body weight change during lactation (g)	0	5	20	80
LD 1-4	12	7	12	18
LD 4-7	7	9	8	5
LD 7-14	21	20	16	18
LD 14-21	2	7	9	9
LD 21-28	-34	-35	-33	-33
LD 1-28	8	8	12	16

F2 generation adverse effects on development

The mean live litter size, percentage of males per litter at birth and postnatal survival between birth and PND 0 (relative to number born), PND 0-1, PND 1-4 (pre-selection), PND 4-7 (post-selection), PND 7-14, PND 14-21, PND 21-28, birth to PND 4 (pre-selection), and PND 4 (post-selection) to PND 28 were unaffected by the test substance at all exposure levels. The number of pups (litters) found dead during PND 0 through to the selection of the F2 generation was 9(7), 14(7), 9(7) and 13(8) in the control, 5, 20 and 80 ppm groups, respectively. A female in the control group and a female in the 80 ppm group had total litter losses between PND 0-2.

F2	0	5	20	80
The mean number of pups born	12.2	14.4*	13.7	12.5
Live litter size	12	14.2*	13.6	12.3
Percentage of males per litter at birth	49.3	53	46.5	47.2
Postnatal survival between birth and PND 0	98.6	98.7	99	98.5
Postnatal survival PND 0-1	95.7	98.9	99.2	96.6
Postnatal survival PND 1-4	99.1	98.8	99.4	95.7
Postnatal survival PND 4-7	99	100	99.5	100
Postnatal survival PND 7-14	100	99	98.7	99.5
Postnatal survival PND 14-21	100	100	100	100
Postnatal survival PND 21-28	100	99.5	99.5	100
Postnatal survival from birth to PND 4	93.4	96.5	97.7	93.7
Postnatal survival PND 4-28	99	98.5	97.7	99.5

According to the DS, the mean F2 pup body weight gain in test substance-exposed groups was similar or slightly lower during the period of suspended F1 maternal exposure (PND 1-4) and following re-initiation of F1 maternal exposure (PND 4-7, 7-14, 14-21, 21-28 and 4-28) when compared to the concurrent control group values. Minor differences from control were not statistically significant (during PND 1-4 and 4-28), and according to the DS did not demonstrate an exposure-related relationship. Mean F2 pup body weights were also reported to be similar or slightly lower (not statistically significant) during the entire lactation period when compared to the concurrent control group. The mean body weight values in the male and female concurrent control group were higher than the mean values in the WIL historical control data for inhalation studies at PND 1 (2.8% and 4.5%, respectively) and at PND 28 (5.3% and 7.0%, respectively).

F2 pup bw gain (m/f)	0	5	20	80

PND 1-4	2.9/2.8	2.6/2.6	2.8/2.7	3.0/2.9
PND 4-7	4.0/3.8	3.6/3.5	3.7/3.5	3.6/3.5
PND 7-14	12.1/11.9	12.6/12.4	12.4/12.2	11.3/11.2
PND 14-21	16.0/15.3	13.3/12.9	13.5/13.2	14.6/14.2
PND 21-28	37.5/33.7	34/30.7	35.8/32.3	35.3/31.4
PND 4-28	69.5/64.8	63.5/59.5	65.4/61.2	64.7/60.3

In the PND 28 necropsy of F2 weanlings selected for organ weights, internal findings included cyst(s) on right kidneys in 1 pup in each of the control and 5 ppm groups, dilated pelvis in the kidney in 1 pup in each of the 5 and 80 ppm groups and dark red areas in the thymus in 1 pup in each of the control, 5 ppm and 80 ppm groups. According to the DS, no test article-related effects on organ weights (absolute, relative to final body weight and relative to brain weight) were observed at any dosage concentration when the test article-exposed groups were compared to the control group.

F1 maternal toxicity

According to the DS, the mean F1 maternal body weight gains in the 80 ppm group were statistically significantly decreased ($p<0.05$) when the entire gestation period (days 0-20) was evaluated as a result of lower ($p<0.01$) mean body weight gain during gestation days 17-20 compared to the control group. Mean body weights in this same group were 13.2% to 15.1% lower than the control group values throughout gestation. The differences from the control group values were statistically significant ($p<0.01$). These mean body weight decreases noted during gestation were a continuation of the decreases observed in this group during the pre-mating period. Mean maternal body weights, body weight gains and cumulative body weight changes in the 5 and 20 ppm groups were unaffected by test article administration. Increased (statistically significant, $p<0.05$) mean body weight gains in the 5 and 20 ppm groups were noted during gestation days 14-17.

F1 maternal body weight change during gestation (g)*	0	5	20	80
GD 0-4	19	18	17	18
GD 4-7	10	10	10	8
GD 7-11	18	20	18	15
GD 11-14	12	12	14	12
GD 14-17	23	28*	28*	22
GD 17-20	41	44	41	33*
GD 0-20	122	132	128	108*

* nongravid weight(s) not included in calculation of mean

A statistically significantly ($p<0.01$) increased mean body weight gain was observed in the 80 ppm group during lactation days 1-4 when compared to the control group and was considered

related to the cessation of inhalation exposure during this time. The increase in weight gain during lactation days 1-4 influenced the weight gain during the overall lactation period (lactation days 1-28) to be statistically significantly increased ($p<0.01$) when compared to the control group.

Statistically significantly ($p<0.01$) lower mean body weight was also noted in the 80 ppm F1 female group during the entire lactation period when compared to the control group. This difference from the control group was attributed to the lower mean body weight gains during the pre-mating period. Mean body weights and mean body weight gains in the 5 and 20 ppm groups were unaffected by test article exposure.

F1 maternal body weight change during lactation (g)	0	5	20	80
LD 1-4	9	9	15	22**
LD 4-7	3	-1	4	3
LD 7-14	18	23	20	19
LD 14-21	5	4	3	2
LD 21-28	-30	-22	-29	-28
LD 1-28	4	12	12	19**

**Significantly different from the control group at 0.01 using Dunnett's test

The slight, not dose-dependent effects on the body weight of F1 and F2 offspring, on the age of attainment of balanopreputial separation in male offspring, and on the mean age of attainment of vaginal patency in F1 offspring are not considered treatment-related. They are considered to be within normal biological variability for these parameters since the values were within the historical control range. The observed effects do not meet the classification criteria either for developmental toxicity or for effects on or via lactation.

2) A reproduction/developmental toxicity screening test (Nemec, 2006c)

Effects on development

The mean number of pups born and live litter size on PND 0 were slightly lower in the 150 ppm group, as shown in a table below, as a result of a single female, which also had an atypically low number of implantation sites. No relationship to the test article was evident. No test article-related effects on the mean number of pups born, live litter size and the percentage of males at birth were observed in the 5 and 50 ppm groups. Postnatal survival in the 150 ppm group was lower during PND 4-28 due to a single female that lost 5 pups during PND 5-9. There were no effects of maternal exposure to the test article on postnatal survival in the 5 and 50 ppm groups.

	0 ppm	5 ppm	50 ppm	150 ppm
Number born	14.5	15.6	15.3	12.9
Postnatal survival on PND 0 (relative to number born)	95.7	97.9	99.5	99.1

Postnatal survival on PND 0 (relative to number born) pre-selection	100	99.1	98.3	99.5
Postnatal survival on PND 0 (relative to number born) post-selection	99.1	100	100	97.3
Postnatal survival on PND 7-14 (relative to number born)	100	100	98.3	97.1
Postnatal survival on PND 14-21 and 21-28 (relative to number born)	100	100	100	100
Postnatal survival on PND 0-4 (relative to number born) pre-selection	95.7	77.1	97.3	98.6
Postnatal survival on PND 4-28 (relative to number born) post-selection	99.1	100	98.3	95
BW PND 1 m/f	7.5/7.1	6.9/6.8	7/6.7	7.1/6.6
BW PND 4 m/f (before selection)	10.3/9.7	9.7/9.6	9.1/8.7	10.2/9.3
BW PND 7 m/f	13.5/13.1	12.5/12.8	12.3/11.7	13.3/11.9
BW PND 14 m/f	23.9/23.5	23.1/23.4	21.4/**19.9***	22.3/**19.5***
BW PND 21 m/f	38/37.6	36.6/37	34.2/**31.9***	36.4/**31.8***
BW PND 28 m/f	76.8/71.9	72.7/69.8	68.3/**62.8***	72.4/**62.4***
BW PND 35 m/f	131/117	123/112	**107*/95****	**108*/92****

PND - postnatal day
* Significantly different from the control group at 0.05 using Dunnett's test
** Significantly different from the control group at 0.01 using Dunnett's test

Parental toxicity

Effects on mean body weights, body weight changes and food consumption were noted in the 50 and 150 ppm group males throughout the study and in the 150 ppm group females during gestation and lactation. Mean body weights in the 50 and 150 ppm group males were up to 5.5% and 12.6% less than those in the control group, respectively, during study weeks 1-4. Mean body weights in females at 150 ppm were up to 12.5% and 10.0% lower than those in the control group during the gestation and lactation periods, respectively. No test substance-related effects on organ weights or macroscopic/microscopic findings were reported.

One female in the 150 ppm group was euthanised *in extremis* on lactation day 7 following a body weight loss of 39g. This female had several clinical findings prior to death, including a pale body and eyes, shallow respiration and red material around the nose and mouth. A cause of death could not be determined at necropsy or microscopically (with a limited number of tissues having been examined). However, the moribund condition of this female was

considered test substance- related since body weight effects were noted for other animals in this group and spontaneous mortality is low in rats, especially during lactation. All other animals survived to the scheduled necropsies. No test substance-related clinical findings were observed in the F0 males and surviving F0 females.

In summary, the exposure to DMDS had no effect on the viability of offspring in the reproduction/developmental toxicity screening test (Nemec, 2006c). The decreases in body weight of pups at 50 and 100 ppm starting in females on 14 PND and in males on PND 35, not being dose-dependent, are most probably related to maternal toxicity since reduced food consumption and body weight were observed in parental animals in these groups. In addition, the results of the lactational inhalation phased-exposure study of DMDS in rats (Nemec, 2006) indicate that DMDS does not induce effects on or via lactation.

3) A prenatal developmental toxicity study in rats (Nemec, 2006d)

Developmental effects

Mean gravid uterine weight at 80 ppm was statistically significantly ($p<0.01$) lower than that of the control group value. Mean foetal weight in the 80 ppm group (3.0 g) was lower than that of the concurrent control group value (3.7 g) and the minimum mean value in the WIL historical control data (3.4 g). The difference from the concurrent control group was statistically significant ($p<0.01$).

Post-implantation loss, live litter size and foetal sex ratios in the 80 ppm group were unaffected by maternal test article exposure. Intrauterine growth and survival were unaffected by the test article at 5 and 20 ppm.

	0	5	20	80
Post-implantation loss	33	22	25	20
Mean foetal weight (g)	3.7	3.5	3.6	3.0**
Male sex ratio	49.3	43.8	55.1	53.3
Viable fetuses	405	405	406	408
Viable fetuses (%)	92.2	94.6	94.3	95.5

** statistically significant ($p<0.01$)

The numbers of foetuses (litters) available for morphological evaluation were 405(27), 405(26), 406(27) and 408(26) in the control, 5, 20 and 80 ppm groups, respectively. Malformations were observed in 2(2), 1(1), 2(2) and 2(1) foetuses (litters) in these same respective exposure groups.

External malformations were noted for 1, 0, 1 and 2 foetuses in the control, 5, 20 and 80 ppm groups, respectively, and included the following:
Microphthalmia (left orbit appeared smaller than normal) in one foetus in the control
group and two foetuses in the 80 ppm group. The other of these latter ones also had anal atresia and vertebral agenesis (all vertebrae posterior to lumbar vertebra no. 4 absent).

The only other external malformation observed in this study, a localised foetal oedema (neck and thorax), was noted in one foetus in the 20 ppm group.
No external developmental variations were noted for foetuses in this study.

Visceral malformations consisted of hydrocephaly (increased cavitation of both lateral ventricles and the third ventricle) in one control group foetus, and a malpositioned oesophagus (located to the right of the trachea) and lobular dysgenesis of the lungs (all right lobes were fused) in one foetus in the 5 ppm group.

No soft tissue malformations were noted in the 20 and 80 ppm groups.
Visceral developmental variations were only noted in the 5 and 20 ppm groups consisting of renal papillae not developed (Woo and Hoar grade 0) and/or distended ureters, and an accessory spleen. Renal papillae not fully developed (Woo and Hoar grade 1) was observed in one foetus in the control, 5 and 20 ppm group. Atrial cysts and a white area on the right atrium were noted in the control group.

The only foetal skeletal malformation in this study, sternoschisis (sternal band nos. 1-6 not joined), was noted for a single foetus in the 20 ppm group.

Test substance-related differences in the mean litter proportions of skeletal developmental variations were noted in the 80 ppm group. These differences included increased mean litter proportions of unossified sternebrae nos. 5 and/or 6, unossified sternebrae nos. 1, 2, 3 and/or 4, reduced ossification of the vertebral arches, unossified pubis and unossified hyoid, and a decreased mean litter proportion of ossified cervical centrum no. 1 at 80 ppm.
Only the difference for unossified sternebrae nos. 5 and/or 6 was statistically significant ($p<0.01$) compared to the concurrent control group. These effects indicating delayed foetal development were considered to be of low or minimal toxicological significance and appeared to be secondary to moderate maternal toxicity, since lower mean maternal body weight gains and food consumption were observed in the 80 ppm group. Therefore the observed delayed foetal development does not warrant classification.

Mean litter proportions of the test article-related skeletal developmental variations:

Finding	0 ppm	5 ppm	20 ppm	80 ppm	WIL HC Mean (Range)
Sternebrae # 5 and/or 6 unossified	22.2	18.3	23.4	51.0++	7.4 (0.3-23.1)
Sternebrae # 1, 2, 3 and/or 4 unossified	0.2	0.2	0.5	4.3	0.2 (0.0-1.3)
Cervical centrum # 1 ossified	26.5	21.6	22.1	15.5	19.4 (6.6-34.4)
Reduced ossification of the vertebral arches	0.2	0.0	0.5	2.2	0.1 (0.0-1.1)
Pubis unossified	0.0	0.0	0.2	0.7	0.1 (0.0-2.3)
Hyoid unossified	0.2	0.2	0.0	0.7	1.5 (0.0-4.2)

HC = Historical control
++ = p0.01

Maternal toxicity

All females in the control, 5, 20 and 80 ppm groups survived to the scheduled necropsy on gestation day 20. No test substance-related clinical findings were noted at the daily examinations, at the midpoint of exposure or 1 hour following the exposure period at any

dosage level.

A test substance-related mean body weight loss during gestation days 6-9 and lower mean body weight gains during gestation days 9-12, 12-20 and 6-20 (the entire exposure period) were noted in the 80 ppm group compared to the control group; the differences were statistically significant (p<0.01).

The decrease in body weight gain throughout the exposure period resulted in mean body weights that were 5.6% to 9.5% lower (p<0.01) than control group values during gestation days 10-20. Also in the 80 ppm group, mean net body weight was 8.5% lower and mean net body weight gain was lower compared to control group values; the differences were statistically significant (p<0.01).

Mean gravid uterine weight at 80 ppm was also statistically significantly (p<0.01) lower than the control group value, corresponding to lower mean foetal weights observed in this group. In the 20 ppm group, mean body weight gains were slightly lower than in the control group during gestation days 6-9 and 9-12; the differences were statistically significant (p<0.05 or p<0.01).

Mean body weight gains in the 20 ppm group were similar to control group values during gestation days 12-20 and when the entire exposure period (gestation days 6-20) was evaluated. Mean net body weight, net body weight gain and gravid uterine weights in this group were similar to control group values.

Mean maternal body weights, body weight gains, net body weight, net body weight gain and gravid uterine weight in the 5 ppm group were unaffected by test substance exposure.

Maternal body weight (g) during gestation	0	5	20	80
Gravid uterine weight	84.8	86	84	73**
BW GD 0	259	261	261	261
BW GD 7	294	299	296	294
BW GD 14	323	326	318	301**
BW GD 20	400	406	395	362**
Corrected BW GD 20	315.2	320	311	289

** statistically significantly (p<0.01)

RAC concludes that DMDS in the prenatal developmental toxicity study in rats via inhalation exposure of pregnant rats (Nemec, 2006d) did not affect viability or induce structural abnormalities of foetuses. Lower foetal weight and increased frequency of delayed ossification in the 80 ppm group are considered of low significance that were most probably linked with lower food consumption and reduction in body weight gain of dams exposed to DMDS at this concentration. Therefore, these effects do not warrant classification.

4) A prenatal developmental toxicity study in rabbits (Nemec, 2005a)

Developmental effects

Intrauterine growth and survival were unaffected by the test substance administration at exposure levels of 15, 45 and 135 ppm.

	0	15	45	135
Post-implantation loss	12	9	4	3
Viable fetuses	213	210	210	218
Fetal BW	41.2	43.6	42.2	40.9
Male sex ratio (%)	46	49	44	51
Small or absent gallbladder (fetuses)	1/213	2/210	1/210	6/218
Small or absent gallbladder (litters)	1/23	1/24	1/23	5/24
Percent per litter with external variations	0	0	0	0
Percent per litter with soft tissue variations	14.6	15.5	24.5	16.5
Percent per litter with skeletal variations	53.9	68.8	77.1**	79.6**
Percent per litter with variations	58.7	73.1	79.1*	83.7**

\+ = Significantly different from the control group at 0.05
++ = Significantly different from the control group at 0.01

There were no external malformations or developmental variations noted in the 15, 45 and 135 ppm groups. In the control group, two foetuses had umbilical herniation of the intestine (several loops of the intestine protruded through an opening in the umbilicus).

Soft tissue malformations were observed in 3(3), 2(1), 3(1) and 3(2) foetuses (litters) in the control, 15, 45 and 135 ppm groups, respectively. One, one, three and two foetuses in the same respective groups had lobular agenesis of the lungs (absent right accessory lobe). One foetus in the control, 15 and 135 ppm groups had hydrocephaly (increased cavitation of both lateral and third ventricles). One foetus in the control group had lobular

dysgenesis in the lungs (all lobes small; right lobes fused).

Soft tissue developmental variations occurred in all groups, including the control group, and consisted primarily of blood vessel variations (the left carotid artery arose from the brachiocephalic trunk, right subclavian artery coursed retroesophageal rejoined aortic arch adjacent to ductus arteriosis with no brachiocephalic trunk, right carotid and subclavian arteries arose independently from the aortic arch with no brachiocephalic trunk), accessory spleens, retrocaval ureter, and small or absent gallbladder (not statistically different from the frequency in the concurrent control group.) Other soft tissue developmental variations observed in the test substance-exposed groups occurred infrequently, they occurred similarly in the control group or the values were within the range of WIL historical control data.

Skeletal malformations were observed in 6(3), 3(3) and 2(2) foetuses (litters) in the control, 15 and 45 ppm groups, respectively. Two, two and one foetus in the control, 15 and 45 ppm groups, respectively, had vertebral anomalies with or without associated rib anomalies consisting of absent and extra arches, centra and/or ribs, mislocated centra and fused ribs and centra. Rib anomalies consisting of extra, fused or forked ribs, were noted for three and one foetus in the control and 45 ppm groups, respectively. One foetus in the 15 and 45 ppm groups had skull anomalies consisting of medially fused nasal or frontal bones. One control group foetus had a costal cartilage anomaly (bifurcated right costal cartilage with the posterior fork associating with the sternum, causing subsequent costal cartilages to associate with the sternum higher than normal). None of the proportional values were statistically significant compared to the control group and the values were within the WIL historical control data ranges.

No skeletal malformations were noted at 135 ppm. The percent per litter of 13th full ribs in the 45 and 135 ppm groups (44.8% and 55.5% per litter, respectively) were increased compared to the control group (31.3% per litter). Although the difference was statistically significant ($p<0.05$) for the 135 ppm group, the values were within the WIL historical control data range (19.4% - 59.1% per litter). Additionally, the percent per litter value of the 7th cervical ribs in the 45 ppm group (7.9% per litter) exceeded the maximum mean value in the WIL historical control data (7.7% per litter); however, this increase did not occur in an exposure-related manner (3.1% per litter in the 135 ppm group).

The increased incidence of supernumerary ribs is a relatively common finding in standard teratology bioassays[1], and previous studies have indicated a possible correlation between their occurrence and general maternal stress. A significant linear relationship between maternal weight loss during treatment and an increase in supernumerary ribs was also noted[2]. The supernumerary ribs in the rat may be considered as a result of developmental delays in a labile region of the axial skeleton and not as a manifestation of a teratogenic event[3]. Other skeletal developmental variations occurred in all groups, including the control group, and consisted of sternebra (e) nos. 5 and/or 6 unossified, bent hyoid arches, 13th rudimentary rib(s), accessory skull bones and 27 presacral vertebrae. The mean litter percent of the skeletal variants observed in the exposure groups occurred similarly to the control group or they were within the range of the WIL historical control data.

Maternal toxicity

Mean body weights, body weight gains, net body weights, net body weight gains and gravid uterine weights in the 15, 45 and 135 ppm groups were unaffected by the test substance-

exposure throughout gestation (days 6-10, 10-14, 14-21, 21-29 and 6-29).

One, three, six (including the female that aborted) and six females in the control, 15, 45 and 135 ppm groups, respectively, had dark red discoloration of or dark red areas on the lungs (generally all lobes).

RAC concludes that DMDS inhaled by pregnant rabbits in this prenatal developmental toxicity study (Nemec, 2005a) at 15, 45 and 135 ppm did not affect viability or growth or induce structural abnormalities of foetuses above the historical control range. The increased number of skeletal variations, such as 13th full ribs, reflect reversible delayed development that do not provide sufficient evidence for classification. Therefore, RAC concludes that the results of this study do not provide evidence warranting classification of DMDS as a developmental toxicant.

5) A prenatal developmental toxicity study in rats (Barker, 1991)

Developmental effects
Litter and foetal weights were reduced at 50 ppm. No malformations were observed in foetuses from the treated groups. A slightly higher incidence of retarded ossification was observed at 50 ppm. This and the lower foetal weight demonstrating foetal growth retardation at 50 ppm were associated with maternal toxicity.

	0 ppm	5 ppm	15 ppm	50 ppm
% pre-implantation loss	11.7	19.1	12.2	15.6
Number of early intrauterine deaths	5	12	13	10
Mean number per female	0.2	0.8	0.5	0.5
Number of late intrauterine deaths	1	0	2	0
Number of dead foetuses	0	0	0	0
% post-implantation loss	2.3	6.9	4.8	4.1
Number of male foetuses	128	73	152	119
Number of female foetuses	130	89	143	115
%male foetuses	49.6	45.1	51.5	50.9
Mean litter weight	43.7	41.8	41.7	38.8
Mean foetal weight	3.8	3.9	3.7	**3.5****
Mean foetal weight males only	4.0	4.1	3.8	**3.6****
Mean foetal weight females only	3.7	3.7	3.6	**3.4****

** significantly different from control at 0.01 by non-parametric ANOVA and Wilcoxon rank-sum test

EXTERNAL AND VISCERAL DEFECTS				
Number of foetuses examined	258	162	295	234
Number showing malformations	1	0	0	0
% of foetuses examined	0.4	0.0	0.0	0.0
Number showing	52	27	65	52

variations				
% of foetuses examined	20.2	16.7	22.0	22.2
SKELETAL DEFECTS				
Number of foetuses examined	136	85	155	121
Number showing malformations	1	0	0	0
% of foetuses examined	0.7	0.0	0.0	0.0
Number showing variations	123	74	139	116
% of foetuses examined	90.4	87.1	89.7	95.9
Total number of foetuses showing malformations	1	0	0	0
% of foetuses examined	0.4	0.0	0.0	0.0

Maternal toxicity

There were no deaths. A higher incidence of rough hair coat was observed at 50 ppm as compared to controls. Clinical condition at 5 and 15 ppm did not differ from controls. At 50 ppm and 15 ppm, the weight gain was 40% and 16% lower than in controls over the exposure period (day 6 to 15, $p < 0.001$). Food intake was lower ($p < 0.001$) than in controls at 50 ppm but comparable to controls at 5 or 15 ppm. No unusual lesions were observed at necropsy.

RAC concludes that inhalation exposure of female rats to DMDS during days 6 to 15 of pregnancy at concentrations 0, 5, 15 or 50 ppm for 6 hours daily did not affect viability or induce structural abnormalities of the foetuses. The slight reduction in weight of foetuses and slightly higher incidence of retarded ossification at 50 ppm were due to delayed foetal development being a secondary, nonspecific consequence of lower food consumption and reduced body weight gain of pregnant females in this group. These effects as such are considered to be of low or minimal toxicological significance that do not warrant classification for developmental toxicity, and they are related to moderate maternal toxicity.

Therefore, RAC concludes that the results of this study do not provide evidence warranting classification of DMDS as developmental toxicant

Conclusions on classification for reproductive toxicity

Effects on fertility and sexual function

Taking into account that no treatment-related, adverse effects on sexual function and fertility were observed in relevant and acceptable studies, RAC is of the opinion that DMDS **does not warrant classification for effects on fertility and sexual function**.

Effects on development

Since in five animals studies on rats and rabbits no adverse effects on viability, frequency of malformations or physical development of offspring were observed, RAC is of the opinion that DMDS does not warrant classification for effects on development. Slight reductions in body weight or body weight gains of pups or delayed ossification at the top doses were considered to be related to moderate maternal toxicity consisting of reduced food consumption and reduced maternal body weight. These effects as such are considered to be of low or minimal

toxicological significance **not warranting classification for developmental toxicity**.

Adverse effects on or via lactation

The existing data on DMDS do not meet not meet the following classification criteria for effects on or via lactation: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one- or two-generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk. Therefore RAC is of the opinion, that DMDS **does not warrant classification for adverse effects on or via lactation**.

10.11 Specific target organ toxicity-single exposure

Table 22: Summary table of animal studies on STOT SE

Method	Results	Remarks	Reference
rat (Sprague-Dawley) male/female inhalation: vapour (whole body) Evaluation of the potential toxic effects of dimethyl disulphide (DMDS) on tissues of the upper respiratory tract (URT) when administered 6 hours/day as a vapor via whole-body inhalation to rats for 1 day or 5 consecutive days.	BMD10% for nasal irritation (severity 2) (6 h): 19 ppm (male/female) (70 (53 - 110) mg/m3. Use of severity 2 would be consistent with a determination that minimal effects are not adverse.)	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Kirkpatrick DT (2008) Haber L, Parker A and Dourson M (2008)
rat (Sprague-Dawley) male inhalation: vapour (whole body) Evaluation of the potential toxic effects of dimethyl disulphide (DMDS) on tissues of the upper respiratory tract (URT) when administered 24 hours as a vapor via whole-body inhalation to rats.	NOAEC for nasal irritation (24 h): 12.5 ppm (male) (48 mg/m3)	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Kirkpatrick DT (2009)

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

In a key study, the potential toxic effects of dimethyl disulfide (DMDS, purity 99.9%) on tissues of the upper respiratory tract (URT) was evaluated when administered as a vapour via 6-hour whole-body inhalation to rats for 1 day or 5 consecutive days at target concentrations of 0, 50, 150, 300 and 600 ppm (192, 577, 1154 and 2318 mg/m^3) (Kirkpatrick, 2008). Clinical examinations were performed daily and detailed physical examinations were performed prior to necropsy. Individual body weights were recorded pretest, prior to the first, third and fifth exposures and prior to necropsy. Ten animals/sex/group were killed approximately 24 hours after a single exposure whilst the remaining 10 animals/sex/group were killed approximately 24 hours after the 5thexposure. Complete necropsies were conducted on all animals, selected organs were weighed and selected tissues were examined microscopically from all animals. No clinical findings attributed to test

article exposure were noted at any exposure concentration. Lower mean body weight gains or mean body weight losses were noted in males of all DMDS-exposed groups throughout the study and in females of all groups during study days 0-2. These deficits in body weight gain resulted in lower mean body weights for males in all groups and females in the 300 and 600 ppm groups on study days 2 and 4. Test article-related pathology effects included histopathologic changes in the nasal epithelia and turbinate bones and organ weight changes in the lung. After a single exposure, acute inflammation and degeneration of the transitional and olfactory epithelia and acute inflammation of the respiratory epithelium were noted at all DMDS exposure concentrations, and degeneration of the respiratory epithelium was noted at concentrations of 150 ppm and higher. These changes were generally exposure concentration-related at a given nasal level, and changes in the respiratory and olfactory epithelia generally lessened in incidence and severity in more caudal nasal sections. After 5 consecutive days of exposure, mean absolute lung and lung/body weights were higher in the 300 and 600 ppm group females. Hyperplasia of the squamous epithelium was noted in ≥300 ppm group males and all DMDS-exposed group females. Hyperplasia of the transitional and respiratory epithelia and degeneration and regeneration of the olfactory epithelium were noted at all test article exposure concentrations in both sexes. Degeneration and acute inflammation of the transitional and respiratory epithelia, and acute inflammation of squamous and olfactory epithelia, were more variable after the 5-day exposure; DMDS-related effects were noted as acute inflammation of the squamous epithelium (50, 300 and 600 ppm group males and ≥300 ppm group females), transitional epithelium (≥150 ppm group males and all test article exposure concentrations in females), respiratory epithelium (≥300 ppm group males and ≥150 ppm group females) and olfactory epithelium (≥50 ppm group males and ≥150 ppm group females), and degeneration of the respiratory epithelium (≥600 ppm). Fibro-osseous proliferation of the bones of the nasal turbinates was observed at exposure concentrations of 150 ppm and higher in males and 300 ppm and higher in females; this finding may be secondary to inflammation rather than a direct effect of DMDS. The presence of regeneration of the olfactory epithelium despite continued exposure suggested that the olfactory epithelial degeneration may be reversible once exposure is discontinued. Based on the results of this study, toxicity of DMDS vapours administered via 6-hour whole-body inhalation to Crl: CD(SD) rats for 1 or 5 days was observed at exposure levels of 50, 150, 300 and 600 ppm as evidenced by body weight deficits, histopathologic changes in the nasal epithelia (acute inflammation, degeneration and/or hyperplasia) and turbinate bones (fibro-osseous proliferation at 150 ppm and higher, probably secondary to inflammation), and organ weight changes in the lung (300 and 600 ppm females after 5 consecutive days treatment only). Regeneration of the olfactory epithelium despite continued exposure indicated possible recovery after cessation of exposure. The no-observed-adverse-effect concentration (NOAEC) for DMDS vapours administered via whole-body inhalation to Crl: CD(SD) rats for 1 or 5 consecutive days was less than 50 ppm. A BMD10% of 19 ppm (73 mg/m^3) (with a lower limit at 95% of 9.3 ppm (35.8 mg/m^3)) was calculated for the nasal irritation (Haber et al., 2008).

In a key study, dimethyl disulphide (purity 99.5%) vapors were administered via a 24-hour whole-body inhalation to4 groups of 10 male Crl: CD(SD) rats(Kirkpatrick, 2009). Target exposure concentrations were 5, 9, 12.5, and 18 ppm (19, 34, 48 and 69 mg/m^3) and a concurrent control group was exposed to filtered air on a comparable regimen. One day following the 24-hour exposure, all animals were euthanized. All animals were observed daily for clinical signs and mortality whilst detailed physical examinations were performed prior to the scheduled necropsy. Individual body weights were recorded prior to the study day 0 exposures and prior to the scheduled necropsy. Complete necropsies were conducted on all animals, and kidneys, liver and lungs (prior to inflation) were weighed at the scheduled necropsy. Nasal tissues at 6 levels were examined microscopically from all animals. All animals survived to the scheduled necropsy. No clinical, body weight or macroscopic findings attributed to test substance exposure were noted at any exposure concentration. Test substance exposure-related degeneration of the olfactory epithelium was observed in Nasal Levels II-VI at exposure concentrations of 12.5 and 18 ppm, and in Nasal Levels III, IV, and V at an exposure concentration of 9 ppm. Degeneration of the olfactory epithelium was also observed in a single nasal level for 2 of 10 control group animals. The degeneration in the affected nasal sections in the 9 ppm group animals consisted of single or very few discrete, extremely small foci that consisted of cellular vacuolation and individual cell pyknosis, without sloughing. Changes in the 12.5 ppm group were also discrete areas comprising a very small percentage of the olfactory epithelium. These changes would be completely reversible and without clinical consequences and were not considered adverse. Degeneration of the olfactory epithelium was observed in 3 or more nasal levels in all 10 animals and was considered adverse

in the 18 ppm group. This conclusion was based on the extent and severity of degeneration including the amount of damaged epithelium with sloughing of sensory and sustentacular cells. The lesions in this group were considered reversible. Slightly higher incidences of inflammation in the olfactory and respiratory epithelium were noted at a concentration of 18 ppm in Nasal Levels III-V. There were no test substance-related degenerative changes in the respiratory, transitional or squamous epithelium. The no observed adverse-effect concentration (NOAEC) for 24-hour whole-body exposure of DMDS to Crl: CD(SD) rats was 12.5 ppm equivalent to 48 mg/m^3.

10.11.2 Comparison with the CLP criteria

In a study in which rats were exposed to DMDS vapours for a single 24 hour exposure, contact toxicity in the form of respiratory tract (nasal) irritation was identified and the NOAEC determined to be 12.5 ppm equivalent to 48.1 mg/m^3.

10.11.3 Conclusion on classification and labelling for STOT SE

On the basis of the transient respiratory tract irritation and in accordance with Regulation (EC) No 1272/2008 DMDS was classified as Specific Target Organ Toxicity (STOT) Single Exposure (SE) Category 3.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

Potential toxic effects of dimethyl disulphide (DMDS) in the upper respiratory tract (URT) were evaluated when administered to rats as a vapour via 6-hour whole-body inhalation for 1 day or for 5 consecutive days at target concentrations of 0, 50, 150, 300 and 600 ppm (0.192, 0.577, 1.154 and 2.318 mg/L) (Kirkpatrick, 2008).

After a single 6-h exposure, acute inflammation and degeneration of the transitional and olfactory epithelia and acute inflammation of the respiratory epithelium were noted at all DMDS exposure concentrations, and degeneration of the respiratory epithelium was noted at 150 ppm and higher (0.577, 1.154 and 2.318 mg/L). These changes were generally exposure concentration-related at a given nasal level, and changes in the respiratory and olfactory epithelia generally was lower in both the incidence and severity in more caudal nasal sections. All animals survived to the scheduled necropsy.

In the second study, DMDS vapours were administered via 24-hour whole-body inhalation to 4 groups of 10 male Crl: CD(SD) rats (Kirkpatrick, 2009). Target exposure concentrations were 5, 9, 12.5, and 18 ppm (0.019, 0.034, 0.048 and 0.069 mg/L). Complete necropsies were conducted on all animals, and kidneys, liver and lungs (prior to inflation) were weighed at the scheduled necropsy. Nasal tissues at 6 levels were examined microscopically from all animals. All animals survived to the scheduled necropsy. No clinical, body weight or macroscopic findings attributed to test substance exposure were noted at any exposure concentration.

Test substance exposure-related degeneration of the olfactory epithelium was observed

in nasal levels II-VI at 12.5 and 18 ppm (0.048 and 0.069 mg/L), and in nasal levels III, IV, and V at 9 ppm.

Degeneration of the olfactory epithelium was also observed in a single nasal level in 2 of 10 control group animals. The degeneration in the affected nasal sections at 9 ppm consisted of a single or very few discrete, extremely small foci that consisted of cellular vacuolation and individual cell pyknosis, without sloughing. Changes at 12.5 ppm were also discrete areas comprising a very small percentage of the olfactory epithelium.

These changes were completely reversible and without clinical consequences and were not considered adverse. Degeneration of the olfactory epithelium was observed in 3 or more nasal levels in all 10 animals and was considered adverse in the 18 ppm group. This conclusion was based on the extent and severity of degeneration including the amount of damaged epithelium with sloughing of sensory and sustentacular cells. The lesions in this group were considered reversible. Slightly higher incidences of inflammation in the olfactory and respiratory epithelium were noted at 18 ppm in nasal levels III-V. There were no test substance-related degenerative changes in the respiratory, transitional or squamous epithelium. The no observed adverse-effect concentration (NOAEC) for 24-hour whole-body exposure of DMDS to Crl: CD(SD) rats was 12.5 ppm, equivalent to 0.048 mg/L.

In an acute inhalation study, DMDS was tested in Sprague-Dawley rats (5 males and 5 females per dose) via 4-hour whole body vapour exposure, according to the EPA OPPTS 870.1300/ OECD TG 403 (Kirkpatrick, 2005a). The following clinical signs were observed at the dose of 847 ppm (3.26 mg/L) at which there was no mortality:

- immediately following exposure - tremors (in 1/5 females), low arousal (in 1/5 females) and increased difficulty in removing from cage/handling (in 4/5 females),
- over the course of the 8-hour observation interval, females had tremors, low arousal levels and were harder to remove from their cages and to handle.
- slight body weight losses (2 to 7 grams) were noted for one female during the study. All surviving animals met or surpassed their initial (study day 0) body weight by study day 14.

There were no gross findings in any of the surviving animals at the scheduled necropsy.

Taking into account the animal data, the DS proposed classification of DMDS for STOT SE 3, H335 (May cause respiratory irritation).

Comments received during public consultation

One MS supported classification of DMDS as STOT SE 3; H335.

Assessment and comparison with the classification criteria

Oral route

The observation of animals after a single application of DMDS by gavage indicates that the substance is irritating to conjunctivae, intestinal and respiratory mucous membranes

and induces transient depression of the central nervous system, leading in some cases to narcosis and coma.

In the acute toxicity study of Yasso (2015) in rats, DMDS at a non-lethal dose of 50 mg/kg bw induced ataxia, prostration and sagging eyelids, indicating a depressive effect on central nervous system. At 400 mg/kg, prostration, flaccid muscle tone, negative righting reflex, sagging eyelids, ataxia and lethargy were noted in surviving and non-surviving rats. Wetness of the nose/mouth area, lacrimation, irregular breathing, chromorhinorrhea, and red staining of the anogenital area were observed after oral administration of DMDS indicating irritation of conjunctivae, intestinal, nasal and respiratory mucous membranes.

DMDS after single oral administration at dose of 300 mg/kg bw to rats (Pelcot, 2010) was not lethal, but induced hypoactivity, dyspnoea and hypersalivation in all animals 10 minutes after treatment. Hypoactivity persisted in 4/6 animals until 4 hours after treatment. In addition, piloerection and dyspnoea were observed in one animal 3 hours after treatment and in another 4 hours after treatment.

In the acute oral toxicity study (Lombard, 1986) DMDS at doses of 100 and 170 mg/kg bw induced sedation, dyspnea and piloerection between 5 min and 4h post treatment, but not mortality. These symptoms were quickly reversible since no clinical signs were observed from D2 to D15 after treatment. At 290 mg/kg bw sedation, dyspnea, hypotonia, piloerection and mortality (30% of animals) were noted between 5 min and 4h post treatment, but no clinical signs were reported from D2 to D15 after treatment in surviving animals. At 350 mg/kg bw DMDS induced sedation between 5 min and 3h post treatment, but no mortality. No clinical signs were reported from D2 to D15.

At 500 mg/kg sedation, hypotonia, dyspnea and mortality (100%) were observed between 5 and 30 min post exposure.

In the acute oral toxicity study (Gilotti, 2007) DMDS at 56 mg/kg bw, non-lethal to rats, induced wetness of the nose/mouth area, lethargy, sagging eyelids, negative righting reflex, ataxia and wetness of the anogenital area in the males within 2 hours post-dosing. Otherwise, the males appeared normal for the full duration of the study. Females appeared normal throughout the study.

In the acute oral toxicity study (Shapiro, 1985) DMDS at 125 mg/kg bw caused clear reversible behavioural changes in surviving female rats: females did not exhibit any movement on the day of dosing, but appeared to have recovered by the next morning, and did not show any signs of toxicity later on. After a single oral administration of DMDS at 188 mg/kg bw the 4 surviving female rats exhibited signs of excessive salivation and lethargy. The rats exhibited signs of lethargy until day 5 post-dosing. From day 6 through 14 the rats did not exhibit any signs of toxicity.

In summary, these acute toxicity studies indicate that DMDS at non-lethal oral doses induces transient, reversible depression of CNS function from reduced locomotor activity to narcosis and causes irritation of conjunctivae, intestinal and respiratory mucous membranes.

Inhalation route

After 4-hour inhalation exposures at 1188 ppm and 1650 ppm, which were lethal for rats,

(Kirkpatrick, 2005a), DMDS induced rales, which were not heard at a lower, non-lethal concentration of 847 ppm. No microscopic examination was conducted, but at necropsy dark discoloration of the lungs was noted in animals found dead during or immediately following exposure at 1308 and 1650 ppm. These observations indicate that DMDS was irritating to the respiratory system, but since these effects were only observed at lethal concentrations they do not fulfil the classification criteria for STOT SE 3 for respiratory tract irritation. Behavioural alterations consisting of ataxia, impaired mobility and low arousal were noted immediately after exposure in a few exposed females, but not in males.

Single inhalation exposure to DMDS for 6 hours at 150 ppm (0.577 mg/L) and higher (300-600 ppm (1.154-2.318 mg/L)) caused acute inflammation and degeneration of the transitional and olfactory epithelia and acute inflammation of the respiratory epithelium (Kirkpatrick, 2008). The reversibility of these changes was not studied according to the CLH report.

A 5-day exposure for 6 hours/day (Kirkpatrick, 2008) caused an increase in mean absolute and relative lung weight of female rats at 300 and 600 ppm (1.154 and 2.318 mg/L). Hyperplasia of the squamous epithelium was noted in ≥300 ppm (1.154 mg/L) group males and in all DMDS-exposed group females. Hyperplasia of the transitional and respiratory epithelia and degeneration and regeneration of the olfactory epithelium were reported in the CLH report at all test article exposure concentrations in both sexes. Fibro-osseous proliferation of the bones of the nasal turbinates was observed at exposure concentrations of 150 ppm (0.577 mg/L) and higher (1.154 and 2.318 mg/L) in males and at 300 ppm and higher (1.154 and 2.318 mg/l) in females. This finding was considered as secondary to inflammation rather than a direct effect of DMDS (Kirkpatrick, 2008). The NOAEC for DMDS vapours administered via whole-body inhalation to rats for 6 hours for 1 or 5 consecutive days was less than 50 ppm. A BMD_{10} of 19 ppm (73 mg/m^3) (with a lower limit at 95% confidence of 9.3 ppm (35.8 mg/m^3)) was calculated for the nasal irritation based on the findings in this study (Haber *et al.*, 2008).

In a key study (Kirkpatrick, 2009), rats were exposed for 24 hours to DMDS vapour at 5, 9, 12.5, and 18 ppm (19, 34, 48 and 69 mg/m^3) and a concurrent control group was exposed to filtered air on a comparable regimen. One day after the 24-hour exposure, all animals were euthanised. All animals were observed daily for clinical signs and mortality whilst detailed physical examinations were performed prior to the scheduled necropsy. No clinical, body weight or macroscopic findings attributed to test substance exposure were noted at any exposure concentration. Complete necropsies were conducted on all animals, and kidneys, liver and lungs (prior to inflation) were weighed at the scheduled necropsy. Nasal tissues at 6 levels were examined microscopically from all animals. All animals survived to the scheduled necropsy.

Test substance exposure-related degeneration of the olfactory epithelium was observed in nasal levels II-VI at exposure concentrations of 12.5 and 18 ppm, and in nasal levels III, IV and V at an exposure concentration of 9 ppm.

The degeneration in the affected nasal sections in the 9 ppm group animals consisted of single or very few discrete, extremely small foci with cellular vacuolation and individual cell pyknosis, without sloughing. Changes in the 12.5 ppm group were also discrete areas comprising a very small percentage of the olfactory epithelium.

Degeneration of the olfactory epithelium was observed in 3 or more nasal levels in all 10 animals and was considered adverse in the 18 ppm (69 mg/m^3) group. This conclusion was based on the extent and severity of degeneration including the amount of damaged epithelium with sloughing of sensory and sustentacular cells. Slightly higher incidences of inflammation in the olfactory and respiratory epithelium were noted at 18 ppm (69 mg/m^3) in nasal levels III-V. The NOAEC for 24-hour whole-body exposure of DMDS to Crl: CD(SD) rats was 12.5 ppm equivalent to 48 mg/m^3.

The results indicated that a single 24-hour whole body inhalation exposure to DMDS at concentrations of 0,019, 0.034, 0.048 and 0.069 mg/L (5, 9, 12.5, and 18 ppm) induced degeneration of mostly olfactory nasal epithelium at concentrations 0.034, 0.048 and 0.069 mg/L. The study authors noted that there were no test substance-related degenerative changes in the respiratory transitional or squamous epithelium.

In summary, the results of the above studies indicate that a single 24-hour inhalation exposure to non-lethal low DMDS concentrations (0.034, 0.048 and 0.069 mg/L) induced damage of nasal olfactory epithelium (Kirkpatrick, 2009), and much higher but still non-lethal concentrations of 150 - 600 ppm (0.577 mg/L - 2.318 mg/L) for 6 hours caused acute inflammation and degeneration of the transitional and olfactory epithelia and acute inflammation of the respiratory epithelium (Kirkpatrick, 2008).

RAC considers that degeneration of transitional and olfactory nasal epithelium represents significant morphological changes indicating functional damage in the upper respiratory tract, possibly in the form of loss of sense of smell. Olfactory cell degeneration after single exposure to DMDS provides the evidence of appreciable cell death in olfactory epithelium, which may lead to atrophy of this epithelium, which was in fact observed after repeated inhalation exposure to vapour of DMDS (Collins, 1992)**.** RAC concludes that the severity of the observed nasal epithelium changes after single inhalation exposure meets the criteria for STOT SE 1 and 2, listed in point 3.8.2.1.7.3 of Annex I to the CLP Regulation. They were observed at concentrations ≤ 10 mg/L/4h, being thus within the guidance value range for category 1. RAC is of the opinion that DMDS warrants **classification as STOT SE 1; H370 (Causes damage to upper respiratory tract (inhalation)**.

Dermal route

The acute dermal toxicity studies provide evidence that DMDS at non-lethal doses induces transient depression of CNS function.

In the acute dermal toxicity study in rats (Gilotti, 2007) at non-lethal dose of 5000 mg/kg bw, the following clinical signs were noted; instances of wetness and soiling of the anogenital area, chromorhinorrhea, sagging eyelids, emaciated appearance, few faeces, lethargy, ataxia, wet red substance on the anogenital area and the nose/mouth area.

In the second acute dermal toxicity study (Shapiro, 1986) at a non-lethal dose of 2000 mg/kg bw in rabbits within 5 minutes of test material application and for approximately 4 hours thereafter, the following symptoms were observed; heavy breathing, loss of righting reflex, spontaneous spasms, pupillary dilation and constriction, unwillingness to stand, lethargy, excessive salivation and flared nostrils. By 24 hours after dosing, the rabbits appeared active and healthy. Necropsy observations revealed dark foci on all lobes and surfaces of the lungs, pale purple or cloudy discoloration of the spleen

accompanied by rough texture and edges in most rabbits. Slight haemorrhage, dark foci and indentation were noted on the surface of the kidneys of a few animals.

In the acute dermal toxicity study in rabbits (Hazleton, 1985) at a non-lethal dose of 2000 mg/kg bw, apathy and prostration were noted in most of the animals between 15 minutes and 3 hours after the application of the substance. An increase in the spontaneous activity was noted for some animals on the first day of treatment. The behaviour of the animals during the remainder of the observation period was considered normal. No macroscopic lesions were observed at sacrifice.

In the acute dermal toxicity study in rabbits (Haynes, 1988), at a non-lethal dose of 2000 mg/kg bw, the following treatment-related observations were recorded; constriction of the pupils in one male, inflammation of iris in 2 females, nasal discharge in 3 males and 4 females and slow breathing in 2 males shortly after dosing. All animals had recovered within 1 hour of dosing.

In addition, transient narcotic effects were observed in rabbits exposed for 28 days or 13 days by dermal route at 106.3 or 1063 mg/kg bw/day, respectively. The effect disappeared by the end of each daily exposure and was not linked with permanent damage of brain or peripheral nervous tissues since no adverse effects were detected in brain, spinal cord or sciatic nerve of animals in histopathological examination.

The observed effects fulfil the CLP criteria for STOT SE 3; H336. Since the observed narcotic effects were transient in nature, disappearing by the end of daily exposure, they shall not be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure (3.8.2.2.2 of Annex I to the CLP Regulation).

Taking into account observations made during acute and repeated dose toxicity studies, RAC considers that DMDS warrants classification as **STOT SE 3; H336 (May cause drowsiness or dizziness)**.

10.12 Specific target organ toxicity-repeated exposure

Table 23: Summary table of animal studies on STOT RE

Method	Results	Remarks	Reference
rabbit [common species] (New Zealand White [rabbit]) male/female short-term repeated dose toxicity: dermal Coverage: occlusive Vehicle: unchanged (no vehicle) Exposure: 10.63 and 106.3 mg/kg/d	NOEL - systemic toxicity: 10.63 mg/kg bw/day (male/female) no effect NOAEL - systemic toxicity: 106 mg/kg bw/day (male/female) clinical signs - A slight and reversible lethargy after each administration LOAEL - local irritation: 8.5 mg/cm² per day (nominal)	1 (reliable without restriction) key study experimental study **Test material: dimethyl disulfide / 624-92-0 / 210-871-0**	Prinsen MK 1990

Method	Results	Remarks	Reference
groups were treated 5 days/week during a four-week period, whereas the 1063 mg/kg/d group was treated with for 2 1/2 weeks (i.e. 13 days of treatment). (DMDS was administered daily, by dermal occlusive application (6 hours daily) to four groups of albino rabbits.) according to OECD Guideline 410 (Repeated Dose Dermal Toxicity: 21/28-Day Study)	(male/female) dermal irritation - skin irritation observed at all dose levels		
rat (Sprague-Dawley) male/female subchronic (inhalation) (whole body) 10, 50, 150, 250 ppm (38, 192, 577 and 962 mg/m^3) (analytical conc.) Vehicle: unchanged (no vehicle) Exposure: 90 days (6 h/day; 5 d/week) OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)	NOAEC (Systemic toxicity): 10 ppm (analytical) (male/female) LOAEC (Systemic toxicity): 50 ppm (analytical) (male/female) based on: test mat. (Clinical signs of toxicity (soiled fur and piloerection); decreased body weights (↓2-14%), body weight gains (↓17-36%), and food consumption (↓4-15%) in both sexes.) LOAEC (Nasal irritation): 10 ppm (analytical) (male/female) based on: test mat. (Minimal respiratory squamous metaplasia in 3/10 males and 4/10 females)	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Collins CJ (1992)
rat (Fischer 344) male/female subchronic (inhalation: vapour) (whole body) 5, 25, or 125 ppm (19, 96 and 481 mg/m^3) (analytical conc.) Vehicle: unchanged (no vehicle) Exposure: 13 weeks (6 h/day, 5 days/wk) OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)	NOAEC (Systemic toxicity): 5 ppm (analytical) (male) NOAEC (Systemic toxicity): 25 ppm (analytical) (female) LOAEC (Systemic toxicity): 25 ppm (analytical) (male) (Decreased body weight gain) LOAEC (Systemic toxicity): 125 ppm (analytical) (female) (Decreased body weight gain)	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Kim HY, Lee SB, Chung YH, Lim CH and Yu IJ (2006) Kim JC (2009)
rat (Sprague-Dawley) male/female subchronic (inhalation: vapour) (whole body) 5, 20 and 80 ppm (19, 77 and 308 mg/m^3) (analytical conc.) Vehicle: clean air Exposure: 13 weeks (6 hours per	NOAEC (systemic toxicity and neurotoxicity): 20 ppm (analytical) (male) (Lower body weight gains, lower food consumption and lower total session motor activity counts at 80 ppm.) NOAEC (systemic toxicity and neurotoxicity): >= 80 ppm (analytical) (female) (No	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Nemec MD (2006a)

Method	Results	Remarks	Reference
day, 7 days per week) OECD Guideline 424 (Neurotoxicity Study in Rodents)	adverse effect at 80 ppm.) NOAEC (local contact (nasal) irritation): 5 ppm (analytical) (male/female) (Mild to moderate degeneration of the nasal olfactory epithelium at 20 and 80 ppm.)		

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

Dermal exposure

In an OECD 410 study, dimethyl disulphide (DMDS) was administered daily by dermal occlusive application (6 hours daily) to four groups of albino rabbits (Prinsen, 1990). The dose levels applied were 0, 0.01, 0.1, and 1.0 mL/kg bw/day, equivalent to 0, 10.6, 106.3, and 1063 mg/kg bw/day, respectively. Animals were dosed 5 days a week during a 4-week period. After each daily dosing, slight to severe lethargy and/or unconsciousnes were observed in the animals of the 0.1 and 1 mL/kg/d group. The 1 mL/kg bw/d group was sacrificed on day 16 of the study, i. e. after 13 days of treatment due to mortalities. DMDS caused severe skin irritation in all dose groups. During the treatment period, decreased body weights and food consumption were observed in males of the 1 mL/kg/d group. There was no effect on organ weights. There were no findings other than skin irritation upon macroscopic examination. Microscopic examination revealed changes in the skin of those animals that showed signs of irritation. The no-observed adverse effect level for systemic effects was 0.01 ml/kg/day (10.6 mg/kg bw/d).

Inhalation exposure

In an OECD 413 study, groups of 10 rats/sex were exposed by inhalation to DMDS 6 h/day, 5 d/week for 90 days to concentrations of 0, 10, 50, 150, 250 ppm (0, 38, 192, 577 and 962 mg/m^3) (Collins, 1992). The exposure of the 150 ppm group was terminated after 6 weeks and its treatment-free subgroup necropsied 2 weeks later. The remaining groups received a 13 week exposure period followed by 4 weeks for the treatment-free subgroups. The only clinical signs attributable to treatment were salivation, lacrimation or reduced activity during exposures 1 and 2 of the 150 and 250 ppm groups and a low incidence of dyspnea or wheezing in the early part of the study, particularly in the 250 ppm animals at week 1. Functional observation tests indicated no evidence of neurotoxicity. Body weight gains and food consumption were decreased in all treatment groups; this effect was reversible during the recovery period. Hematological profiles suggested a possible small reduction in Hb, RBC and PCV in the 250 ppm female group only. Blood chemistry examinations showed treatment-related changes in ALT, alkaline phosphatase and bilirubin. The changes did not include the 10 ppm group except for elevated ALT in occasional animals at week 13 and after the treatment-free period. There were no changes in organ weights that were considered to be treatment-related and no treatment-related macroscopic abnormalities. Microscopic evaluations indicated a dose-related effect on nasal mucosa characterised by squamous metaplasia of the respiratory epithelium accompanied by atrophy and microcavitation in the anterior olfactory epithelium. In the 10 ppm group the effects were limited to a local, minor degree of squamous metaplasia of the anterior nasal cavity. The changes were still present in the 50 and 250 ppm groups after the treatment-free period but the 10 ppm group was generally unremarkable. Clear treatment-related effects were seen at 50 and 250 ppm and were present to a marginal degree at 10 ppm.

In a study carried out following the OECD guideline # 413, groups of 10 F344 rats of each sex were exposed to dimethyl disulphide (DMDS) vapor by whole-body exposure at concentrations of 0, 5, 25, or 125 ppm (0, 19, 96 and 481 mg/m^3) for 6 h/day, 5 days/wk for 13 wk (Kim et al., 2006). All the rats were sacrificed at the end of treatment period. During the test period, clinical signs, mortality, body weights, food consumption, ophthalmoscopy, urinalysis, hematology, serum biochemistry, gross findings, organ weights, and histopathology were examined. At 25 ppm, a decrease in the body weight gain and food intake was observed

in the males, but not in the females. However, at 125 ppm, a decrease in the body weight gain, food intake, and thymus weight and an increase in the weights of adrenal glands were observed in both genders. In contrast, no treatment-related effects were observed in the 5 ppm group. In these experimental conditions, the target organ was not determined in rats.

In the subchronic neurotoxicity study via the inhalation route conducted with dimethyl disulphide (DMDS) following the OECD guideline # 424 and reported in IUCLID section 7.9.1. (Nemec, 2006), four groups of 12 male and 12 female Crl: CD(SD) BR were exposed to either clean filtered or DMDS vapor atmospheres or 5, 20 or 80 ppm (0, 19, 77 and 308 mg/m^3) for 6 hours daily in whole-body inhalation chambers for 13 consecutive weeks. A sacrifice, a microscopic examination of nasal tissues was performed. Minimal to moderate degeneration of the olfactory epithelium on nasal Level II was observed in all 80 ppm males and females. In general, the olfactory epithelium of the females was more severely affected. Minimal to moderate degeneration of the olfactory epithelium was noted on nasal Level II in 6/6 males and 4/6 females in the 20 ppm group. Olfactory epithelial degeneration, characterized by a loss of the adluminal cytoplasmic layer of the sustentacular cells, was also found on Levels III and IV in the 80 ppm group. Minimal olfactory epithelial degeneration on Levels III and IV was noted in 1/6 males in the 20 ppm group. The only test article-related finding in the 5 ppm group consisted of minimal degeneration of the olfactory nasal epithelium on nasal Level III in 1/6 males. On Level II, degeneration of the olfactory epithelium was most noticeable on the dorsal arches, while on Levels III and IV, the alteration typically affected the medial aspects of turbinates in the dorsal meatus.

10.12.2 Comparison with the CLP criteria

DMDS has been tested in three 90-day repeated dose toxicity studies by inhalation (Collins CJ, 1992 and Kim et al, 2006 and Nemec MD, 2006). Following repeated inhalation exposures of rats to DMDS, contact (nasal) irritation was a significant finding in 2 of the studies (Collins CJ, 1992 and Nemec MD, 2006) with a range of LOAECs from 10 ppm (38 mg/m^3) (Collins CJ, 1992) to 20 ppm (77 mg/m^3) (Nemec MD, 2006). A local NOAEC for irritation of 5 ppm (19 mg/m^3) (Nemec MD, 2006) was identified. Irritation effects in the nasal cavities were always present at a lower concentration than systemic toxicity. Systemic toxicity, based on the most sensitive finding of lower body weights, was present in all 3 studies and in general, males appeared more sensitive to DMDS than females. The LOAECs for this finding ranged from 25 ppm (96.2 mg/m^3) (Kim et al, 2006) to 80 ppm (308 mg/m^3) (Nemec MD, 2006) depending on the dose concentrations selected for the studies and worst case values. On a weight of evidence approach the NOAEC for systemic toxicity based on this finding was 20 ppm (96.2 mg/m^3) (Nemec MD, 2006)

In addition to the inhalation toxicity studies, skin irritation and necrosis were noted in rabbits dosed dermally with DMDS at 1063 mg/kg bw/day for 16 days and 106.3 and 10.6 mg/kg bw/day for 28 days. At 1063 mg/kg bw/day, increased mortality, spasms, and effects on the heart and red blood cells were noted. Transient clinical manifestations on the CNS (lethargy) were noted at 106.3 and 1063 mg/kg bw/day. A NOAEL for systemic toxicity of 106.3 mg/kg bw/day was established, while the overall NOAEL including local skin effects was found to be less than 10.63 mg/kg bw/day (Prinsen, 1990).

10.12.3 Conclusion on classification and labelling for STOT RE

Repeated inhalation exposures of rats to DMDS for 90 days, induced a transient irritation of the nasal passage from 10 ppm, reduced body weight gain from 25 ppm and slight effects on red blood cells and/or some clinical chemistry parameters at 250 ppm. Microscopic changes observed in the noses in all groups were generally reversible after four weeks of non-exposure but not complete at 50 and 250 ppm. In consequence according to CLP criteria, DMDS is not considered to induce significant toxic effects of relevance to human health and don't warrant a classification for specific target organ toxicity-repeated exposure following inhalation exposure.

Repeated dermal administration of 1063 mg/kg bw/day DMDS to rabbits for 16 days induced signs of systemic toxicity (mortality, CNS depression, changes in blood parameters and microscopic changes in the heart). However, a lower dose level, 106.3 mg/kg bw/day administered for 28 days, induced only a transient lethargy after the treatment. In the absence of any signs of histopathological changes in brain, spinal corde

and sciatic nerve in the exposed animals, this transient CNS effect is not considered as a significant toxic effect. In consequence according to CLP criteria, DMDS is not considered to induce significant toxic effects of relevance to human health and don't warrant a classification for specific target organ toxicity-repeated exposure following dermal exposure.

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

Summary of the Dossier Submitter's proposal

The CLH dossier contains several repeated dose toxicity studies of DMDS: one in rabbits (Prinsen, 1990) and three in rats (Collins, 1992; Kim *et al*., 2006; Nemec, 2006).

Dermal exposure

In an OECD TG 410 study, DMDS was administered daily by dermal occlusive application (6 hours daily) to four groups of albino rabbits (Prinsen, 1990). The dose levels applied were 0, 0.01, 0.1, and 1.0 mL/kg bw/day, equivalent to 0, 10.6, 106.3, and 1063 mg/kg bw/day, respectively. Animals were dosed 5 days a week during a 4-week period. After each daily dosing, slight to severe lethargy and/or unconsciousness were observed in the animals of the 0.1 mL/kg bw/day (106.3 mg/kg bw/day) and 1 mL/kg bw/day (1063 mg/kg bw/day) group. The 1 mL/kg bw/day group was sacrificed on day 16 of the study, i. e. after 13 days of treatment due to mortalities. DMDS caused severe skin irritation in all dose groups. During the treatment period, decreased body weights and food consumption were observed in males of the 1 mL/kg bw/day group. There was no effect on organ weights. There were no findings other than skin irritation upon macroscopic examination at 0.01 mL/kg bw/day (10.6 mg/kg bw/day). Microscopic examination revealed changes in the skin of those animals that showed signs of irritation. The no-observed adverse effect level for systemic effects was 0.01 mL/kg/day (10.6 mg/kg bw/d).

Inhalation exposure

In an OECD TG 413 study (Collins, 1992), groups of 10 rats/sex were exposed by inhalation to DMDS 6 h/day, 5 d/week for 90 days to concentrations of 0, 10, 50, 150, 250 ppm (0, 38, 192, 577 and 962 mg/m^3). The exposure of the 150 ppm (577 mg/m^3) group was terminated after 6 weeks and its treatment-free control necropsied 2 weeks later. The remaining groups received a 13-week exposure period followed by a 4-week recovery period. The only clinical signs attributable to the treatment were salivation, lacrimation or reduced activity during exposures 1 and 2 of the 150 (577 mg/m3) and 250 ppm (962 mg/m3) groups and a low incidence of dyspnea or wheezing in the early part of the study, particularly in the 250 ppm (962 mg/m^3) animals at week 1. The functional observation battery tests indicated no evidence of neurotoxicity. Body weight gains and food consumption were decreased in all treatment groups, but this effect was reversible during the recovery period. Haematological profiles suggested a possible small reduction in Hb, RBC and PCV in the 250 ppm (962 mg/m^3) female group only. Blood chemistry examinations showed treatment-related changes in ALT, alkaline phosphatase and bilirubin. These changes were not observed in the 10 ppm group except the elevated ALT occasionally at week 13 and after the treatment-free period. There were no changes in organ weights that were considered to be treatment-related and no treatment-related

macroscopic abnormalities. Microscopic evaluations performed in the 0, 10, 50 and 250 ppm groups revealed a dose-related effect on nasal mucosa characterised by squamous metaplasia of the respiratory epithelium in all DMDS-treated groups accompanied by atrophy and microcavitation in the anterior olfactory epithelium in 50 and 250 ppm groups. In the 10 ppm group the effects were limited to a local, minor degree of squamous metaplasia of the anterior nasal cavity, whereas at 50 and 250 ppm these effects were of a more severe grade. The observed changes were also present in the 50 and 250 ppm groups after the treatment-free period but in the 10 ppm group the observed effects were generally reversed after the treatment-free period. In summary, clear treatment-related effects were seen at 50 and 250 ppm (192 and 962 mg/m^3) and they were present to a marginal degree also at 10 ppm (38 mg/m^3).

In a study (Kim et al., 2006) carried out in accordance with the OECD TG 413, groups of 10 F344 rats of each sex were exposed to DMDS vapour by whole-body exposure at concentrations of 0, 5, 25, or 125 ppm (0, 19, 96 and 481 mg/m^3) for 6 h/day, 5 days/week for 13 weeks. All the rats were sacrificed at the end of treatment period. During the test period, clinical signs, mortality, body weights, food consumption, ophthalmoscopy, urinalysis, haematology, serum biochemistry, gross findings, organ weights and histopathology were examined. At 25 ppm (96 mg/m^3), a decrease in the body weight gain and food intake was observed in the males, but not in the females. However, at 125 ppm (481 mg/m^3), a decrease in the body weight gain, food intake, and thymus weight and an increase in the weights of adrenal glands were observed in both sexes. In contrast, no treatment-related effects were observed in the 5 ppm group. In these experimental conditions, the target organ was not determined in rats.

In the subchronic neurotoxicity study (Nemec, 2006) via the inhalation route conducted with DMDS in accordance with the OECD TG 424, four groups of 12 male and 12 female Crl: CD(SD) BR rats were exposed to either clean filtered or DMDS vapour atmospheres of 5, 20 or 80 ppm (0, 19, 77 and 308 mg/m^3) for 6 hours daily in whole-body inhalation chambers for 13 consecutive weeks. Microscopic examination of the nasal tissues were performed. Minimal to moderate degeneration of the olfactory epithelium on nasal level II was observed in all 80 ppm (308 mg/m^3) males and females. In general, the olfactory epithelium of the females was more severely affected. Minimal to moderate degeneration of the olfactory epithelium was noted on nasal level II in 6/6 males and 4/6 females in the 20 ppm (77 mg/m^3) group. Olfactory epithelial degeneration, characterised by a loss of the adluminal cytoplasmic layer of the sustentacular cells, was also found on levels III and IV in the 80 ppm (308 mg/m^3) group. Minimal olfactory epithelial degeneration on levels III and IV was noted in 1/6 males in the 20 ppm (77 mg/m^3) group. The only test article-related finding in the 5 ppm (19 mg/m^3) group consisted of minimal degeneration of the olfactory nasal epithelium on nasal level III in 1/6 males. On level II, degeneration of the olfactory epithelium was most noticeable on the dorsal arches, while on levels III and IV, the alteration typically affected the medial aspects of turbinates in the dorsal meatus.

Based on the above data, the Dossier Submitter considered that DMDS does not induce significant toxic effects of relevance to human health and does not warrant a classification for specific target organ toxicity-repeated exposure following inhalation and dermal exposures.

Comments received during public consultation

One MSCA agreed with the proposal for no classification for STOT RE noting that based on the presented summaries, the conclusion "conclusive but not sufficient for classification" is supported.

Another MSCA proposed to classify DMDS as STOT RE 1 for skin based on results of the 28-day study on rabbits by the dermal route. The MSCA also provided more details on the results of the study.

Assessment and comparison with the classification criteria

Dermal route

In rabbits exposed repeatedly to DMDS by occlusive application on skin for 6 hours in doses of 0.1 and 1.0 mL/kg bw/day, equivalent to 106.3 and 1063 mg/kg bw/day (Prinsen, 1990) temporary effects on the central nervous system (CNS) were observed. The observed behavioural effects consisted of slight lethargy in the 106.3 mg /kg bw/day group and of distinct to severe lethargy and unconsciousness in the 1063 mg /kg bw/day group. At the end of each daily exposure, these effects were no longer observed. During the four-week test period, treatment-related signs of abnormal behaviour were not observed in the animals of the 10.6 mg/kg bw/day group or in the controls. During the second and third week of the study, treatment-related mortality occurred in males and females of the 1 mL/kg bw/day group (1063 mg /kg bw/day). Therefore, it was decided to discontinue the treatment of the 1 mL/kg bw/day group on nominal day 16 of the study, i.e. after 13 days of treatment. Noting that CNS effects were of a transient nature and histopathological examinations revealed only treatment-related changes in the heart of some males and females of the 1 mL/kg bw/day (1063 mg/kg bw/day) group, but not in other internal organs, it is plausible that the increased mortality in that group could be related to stress and possible haemodynamic changes induced by severe inflammation of a relatively large area of skin at the site of application (15 cm x 15 cm = 225 cm^2), equivalent up to ca. 10% of the rabbit body surface.

DMDS is not acutely toxic by dermal route and does not require classification due to acute dermal toxicity in rats (LD_{50} > 2000 mg/kg bw), however single doses during repeated dermal exposure of rabbits, starting from a dose of 106.3 mg /kg bw/day, induced transient narcotic effects in rabbits, indicating its systemic availability and dermal absorption, although due to the lack of a toxicokinetic study the dermal absorption of DMDS cannot be quantified.

These transient narcotic effects in rabbits exposed for 28 days or 13 days by the dermal route at doses 106.3 and 1063 mg /kg bw/day, respectively, had disappeared at the end of each daily exposure and were not linked with permanent damage of the brain or peripheral nervous tissues since no adverse effects were detected in brain, spinal cord or sciatic nerve of animals in histopathological examinations after termination of repeated exposure. However, since these narcotic effects occurred also during single daily exposure, they fulfil the criteria for STOT SE 3 for narcotic effects.

Mortality and not clearly defined microscopic changes in the heart observed in rabbits of the highest dose group (1063 mg/kg bw/day) did not meet the criteria for STOT RE 2

since they occurred at a dose approximately two-fold higher than 600 mg/kg bw/day, the upper limit guidance value for this category. The absolute and relative organ weights measured at autopsy did not show any statistically significant differences that could be ascribed to the treatment. No treatment-related microscopic changes were found in the brain, spinal cord, sciatic nerve, or thymus at doses within the STOT RE 2 guidance values. The guidance value range for classification in STOT RE 2 via dermal exposure in a 28-day study is $60 < C \leq 600$ mg/kg bw/day. Since no significant adverse effects were seen in organs of rabbits other than skin at doses $60 < C \leq 600$, the study does not provide sufficient evidence to classify DMDS in subcategory STOT RE 2.

The adverse effects on skin of rabbits were severe and increased in severity with as the time of dermal exposure was increased. They varied from very slight, well-defined or moderate erythema, very slight or slight oedema, and ischemic necrosis in first week of dermal exposure to skin incrustation, which almost completely covered the treated skin area in the second week. During the third and fourth week of exposure, the severity of the encrustation in most animals of the three dose groups was such that scoring of erythema and oedema was no longer possible.

DMDS in two standard skin irritation/damage studies (Guillot, 1985a; Shapiro, 1985) induced skin erythema and oedema, however the classification criteria for skin irritation were not fulfilled. In the OECD TG 404 study (Guillot, 1985a) the mean 24, 48 and 72 hour scores were 1.78 and 1.22 for erythema and for oedema, respectively, however the observations were not continued beyond 72 hours, so their reversibility, although probable, was not shown. In the second study performed according to the EPA 40 CFR 163.81-5 (Shapiro, 1985), the mean 24, 48 and 72 hour scores were 1.03 and 0.11 for erythema and for oedema, respectively, and all lesions were reversible within 10 days. Therefore it is concluded that single dermal exposure had produced noticeable skin inflammation in all exposed rabbits, lasting at least for a few days, although their severity did not meet the classification criteria for Skin Irrit. 2. These data from single dermal exposure acute studies did indicate that repeated, occlusive dermal exposure to pure technical DMDS would indeed lead to significant skin irritation and damage over time. RAC also noted that consideration of local skin effects under STOT-RE for classification purposes is not straightforward.

According to CLP (section 3.9.1.1), the target organ toxicity (repeated exposure) does not include other specific toxic effects that are addressed in sections 3.1 to 3.8 and 3.10 of CLP and this includes skin corrosion/irritation. RAC considers that on balance, the skin effects induced by repeated, occlusive dermal exposure to DMDS for 13 or 28 days should not [in this specific case] result in classification for STOT RE. When taking the lack of (acute) skin irritation/corrosion classification and the proposed Skin Sens. 1 classification into account, RAC concludes to not apply any additional warning for the local skin effects.

Inhalation route

In case of a 90-day repeated inhalation exposure to vapour, the guidance value range for STOT RE 1 is ≤ 200 mg/m^3/6h/day, and for STOT RE 2 is 200 mg/m^3/6h/day < C ≤ 1000 mg/m^3/6h/day. These concentration ranges were covered in three reported inhalation studies.

The effects in the 90-day repeated inhalation study, in which rats were exposed at concentrations of 0, 10, 50, 150 and 250 ppm (0, 38, 192, 577 and 962 mg/m^3,

respectively) (Collins, 1992), consisted of salivation, lacrimation or reduced activity during the 1st and 2nd exposures to concentrations of 577 mg/m^3 and 962 mg/m^3 and of a low incidence of dyspnea or wheezing in the early part of the study, particularly at 962 mg/m^3 at week 1. Microscopic evaluations were performed in the 0, 38, 192 and 962 mg/m^3 groups and revealed a dose-related effect on nasal mucosa characterised by squamous metaplasia of the respiratory epithelium in all DMDS-treated groups accompanied by atrophy and microcavitation in the anterior olfactory epithelium in the 192 and 962 mg/m^3 groups. In the 38 mg/m^3 group the effects were limited to local, minor squamous metaplasia of the anterior nasal cavity, whereas at 192 and 962 mg/m^3 these effects were of a more severe grade. The observed changes were still present in the 192 and 962 mg/m^3 groups after the treatment-free period, but in the 38 mg/m^3 group the observed effects were generally reversed after the treatment-free period. In summary, clear treatment-related effects were seen at 192 mg/m^3 and 962 mg/m^3 and they were present to a marginal degree at 38 mg/m^3. No other significant adverse effects were observed in the functional observation battery, haematological examinations, blood chemistry and histopathological examinations at any concentrations.

The effects observed in the subchronic neurotoxicity study via the inhalation route (Nemec, 2006) consisted mostly of degeneration of the olfactory nasal epithelium at concentrations 77 and 308 mg/m^3.

Thus, the adverse effects observed in Collins (1992) and Nemec (2006) studies were confined to the nasal respiratory and olfactory epithelium, initiated already at the beginning of repeated inhalation exposure. These effects are already covered by the classification STOT SE 1; H370: Causes damage to upper respiratory tract (inhalation) due to occurrence of similar changes in upper respiratory epithelium following single inhalation exposure. Therefore it is not justified to classify for the same effects also with STOT RE.

There were no significant adverse effects observed in rats in the 13-week study (Kim *et al*., 2006) with exposure to DMDS by inhalation 6h/day; 5d/week at concentrations of 19, 96 and 481 mg/m^3 justifying classification for STOT RE.

Oral route

There were no studies of repeated dose toxicity by the oral route.

Summary

Via the inhalation route there were no adverse effects other than those in the nasal epithelium observed in rats in the repeated dose studies at concentration ranges of 38-962 mg/m^3. Via dermal exposure in rabbits at $60 < C \leq 600$, there were no adverse effects in organs other than skin. There were no studies of repeated dose toxicity by oral route.

Based on the existing evidence RAC concludes that data are conclusive, but **not sufficient for classification for (STOT RE)**.

10.13 Aspiration hazard

Even if the kinematic viscosity of DMDS is lower than 20,5 mm²/s, DMDS is not an hydrocarbon and is not known to cause human aspiration toxicity hazards. Therefore, no classification is warranted for aspiration toxicity.

RAC evaluation of aspiration toxicity
Summary of the Dossier Submitter's proposal Kinematic viscosity measured according to OECD TG 114 was equal to 0.59 mm^2/s at 20°C and 0.49 mm^2/s at 40°C (Gancet, 2010). According to the DS, DMDS is not known to cause human aspiration toxicity, the kinematic viscosity of DMDS is lower than 20.5 mm²/s, but DMDS is not a hydrocarbon, therefore no classification is warranted for human aspiration toxicity hazard. **Comments received during public consultation** One MSCA indicated that the low kinematic viscosity would support a classification for Asp. Tox. 1. According to the CLP criteria (section 3.10.2 of Annex I to the CLP regulation), classification into category 1 includes but is not limited to hydrocarbons. The DS responded that there is no reported evidence of aspiration hazard in human for DMDS, and although the kinematic viscosity of DMDS is lower than 20.5 mm²/s, DMDS is not a hydrocarbon, therefore classification criteria are not met. **Assessment and comparison with the classification criteria** In line with CLP criteria a substance is classified for aspiration toxicity: (a) based on reliable and good quality human evidence or (b) if it is a hydrocarbon and has a kinematic viscosity of 20.5 mm²/s or less, measured at 40°C. Taking into account that there is no reported evidence of aspiration hazard in human for DMDS, and DMDS is not a hydrocarbon, the classification criteria for category 1 of aspiration toxicity for DMDS are not fulfilled. Therefore RAC is of the opinion that DMDS **does not warrant classification for aspiration hazard.**

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 24: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Test type: ready biodegradability	not readily biodegradable	2 (reliable with	Thiebaud

Method	**Results**	**Remarks**	**Reference**
OECD Guideline 301 D (Ready Biodegradability: Closed Bottle Test) EU Method C.4-E (Determination of the "Ready" Biodegradability - Closed Bottle Test)	% Degradation of test substance: < 10 after 28 d (DOC removal)	restrictions) key study experimental result **Test material (EC name): dimethyl disulphide**	H., Moncel N. (1995)
Test type: ready biodegradability activated sludge, domestic (adaptation not specified) equivalent or similar to OECD Guideline 310 (Ready Biodegradability - CO2 in Sealed Vessels (Headspace Test)	not readily biodegradable % Degradation of test substance: 50 — 60 after 28 d (CO2 evolution)	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	BASF AG (2001a)
Test type: degradation simulation test OECD Guideline 308 (Aerobic and Anaerobic Transformation in Aquatic Sediment Systems)	% Degradation of test substance: <5% after 7.0 h (radiochem. meas.)	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Allan J. (2011)
Test type: degradation simulation test EPA OPPTS 835.3300 (Soil Biodegradation)	% Degradation of test substance: Soil 1: 12.3% after 120 d (Sum of KOH, various soil extracts and PES) Soil 2: 15.4% after 120 d (Sum of KOH, various soil extracts and PES) Soil 3: 42.7% after 120 d (Sum of KOH, various soil extracts and PES) Soil 4: 41.9% after 59 d (Sum of KOH, various soil extracts and PES)	1 (reliable without restriction) key study experimental result **Test material (EC name): dimethyl disulphide**	Conway S. and Ribach P. (2007)
Method	Results	Remarks	Reference
OECD Guideline 111 (Hydrolysis as a Function of pH)	Half-life (DT50): t1/2 (pH 4): > 1 yr at 25 °C t1/2 (pH 7): > 1 yr at 25 °C t1/2 (pH 9): > 1 yr at 25 °C Recovery (in %): pH 4: 107 at 50 °C after 5 d pH 7: 96 at 50 °C after 5 d pH 9: 93 at 50 °C after 5 d Transformation products: no	1 (reliable without restriction) key study experimental result Test material (EC name): dimethyl disulphide Form: liquid	Li F (2006)

11.1.1 Ready biodegradability

Dimethyldisulfide ready biodegradability was investigated in two independent screening tests according to OECD 301D (closed bottle test) and OECD 310 guidelines, both compliant with GLP. In the first study (OECD 301D), dimethyldisulfide was found to be not readily biodegradable, with less than 10% of biodegradation after 28 days. In the second study (OECD 310), dimethyldisulfide was found to be partly biodegradable with a 50 - 60% mineralisation (CO2 evolution) within 28 days. **According to these results, it is concluded that dimethyldisulfide is not readily biodegradable.**

The data are relevant and adequate for classification purposes under CLP EC 1272/2008 regulation.

Data used for classification: not readily biodegradable.

11.1.2 Degradation simulation testing in sediment (aerobic)

Dimethyldisulfide degradability in sediment was investigated under aerobic conditions according to the OECD 308 guideline. The main dissipation mechanism was a very rapid displacement of dimethyldisulfide from sediment to the water compartment and then to the air compartment. No degradation could be observed in the sediment. According to the guidance on the application of the CLP criteria (v4.0, Nov. 2013, section II.2.3.6, p. 586) a substance can be considered rapidly degradable in water if: (i) no pre-adaptation of the sediment microorganisms has taken place and (ii) an environmentally realistic concentration of substance has been tested and (iii) the substance is ultimately degraded within 28 days with a half-life < 16 days. In the absence of any observed degradation in sediment under aerobic conditions in the available study, **it is therefore concluded that this study does not indicate that dimethyldisulfide would be rapidly degradable in the water compartment.**

The data are relevant and adequate for classification purposes under CLP EC 1272/2008 regulation.

Data used for classification: no evidence that dimethydisulfide is rapidly degradable in sediment according to CLP criteria.

11.1.3 Degradation simulation testing in soil (aerobic)

Dimethyldisulfide degradability in soil was investigated under aerobic conditions according to the OPPTS 835.3300 guideline. The main dissipation mechanism was volatilization of dimethyldisulfide from the soil to the air compartment. Although some measurable degradation occurred with methanesulfonic acid and CO2 as major metabolites, no degradation half-life could be determined. According to the guidance on the application of the CLP criteria (v4.0, Nov. 2013, section II.2.3.6, p. 586) a substance can be considered rapidly degradable in water if: (i) no pre-adaptation of the soil microorganisms has taken place and (ii) an environmentally realistic concentration of substance has been tested and (iii) the substance is ultimately degraded within 28 days with a half-life < 16 days. In the absence of any derived degradation half-life and in the absence of total mineralization of dimethyldisulfide within 28 days in soil under aerobic conditions in the available study, **it is therefore concluded that this study does not indicate that dimethyldisulfide would be rapidly degradable in the water compartment.**

The data are relevant and adequate for classification purposes under CLP EC 1272/2008 regulation.

Data used for classification: no evidence that dimethydisulfide is rapidly degradable in the soil compartment according to CLP criteria.

11.1.4 Hydrolysis

Dimethyldisulfide hydrolysis was investigated in one GLP compliant study according to OECD 111 guideline. The objective of this study was to investigate the hydrolysis of dimethyl disulphide (DMDS) in buffered aqueous solutions at pH 4, 7 and 9 at environmental temperature.

As requested by the OECD 111 guideline, a preliminary study was conducted prior to deciding to perform (or not) the definitive study. Dimethyldisulfide stability was tested at three pH (4, 7 and 9) at 50°C for five days. Radiolabelled 14C-DMDS was applied to the test solution at a rate of 14.91ppm. Mass balance

between D0 and D5 in the preliminary test was between 92% to 101% for pH 4 samples, 97% to 112% for pH 7 samples and 90% to 100% for pH 9 samples, indicating that there was no significant loss of radioactivity during the test period.

Concentration of DMDS in samples was measured by HPLC equipped with a radiochemical detector. The concentrations measured at D0 were set at 100% for both total radioactivity and DMDS concentration for each pH level. After 5 days incubation, DMDS recoveries based on Day 0 were 107% at pH 4, 96% at pH 7 and 93% at pH 9. The concentrations for all pH samples therefore showed no significant decrease over the five day interval at 50°C. The data therefore indicate that DMDS did not undergo hydrolysis at 50 °C in the pH 4, 7 and 9 aqueous solutions within the five day test period. Consequently, DMDS is considered as hydrolytically stable (half-life > 1 year) at environmentally relevant pH and temperatures. All validity criteria as set in the OECD 111 guideline were met. **It is concluded that dimethyldisulfide is not hydrolysable at environmental temperatures and pH.**

The data are relevant and adequate for classification purposes under CLP EC 1272/2008 regulation.

Data used for classification: not hydrolysable at environmental temperatures and pH.

11.1.5 Conclusion on rapid degradability according to CLP criteria

According to guidance on the application of the CLP criteria (v4.0, Nov. 2013, section 4.1.3.2.3.2, p. 518), a substance is considered to be not rapidly biodegradable unless at least one of the following is fulfilled:

a. The substance is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability.
b. The substance is demonstrated to be ultimately degraded in a surface water simulation test with a half-life of < 16 days.
c. The substance is demonstrated to be primarily degraded biotically or abiotically in the aquatic environment with a half-life < 16 days
d. The substance is demonstrated to be ultimately degraded in an aquatic sediment or soil simulation test with a half-life < 16 days and the ratio BOD5/COD is superior to 5 if only those data are available.
e. A weight of evidence approach based on read-across provides convincing evidence that the substance is rapidly degradable.

Dimethyldisulfide is not readily biodegradable, not hydrolysable at environmental temperatures and pH and no convincing evidence of rapid degradation was noted in any degradation study in soil and sediment under aerobic conditions. In addition, no degradation simulation study is available for surface water. To our knowledge, no read-across or *in silico* model for rapid degradation is available and relevant for dimethyldisulfide.

Conclusion on rapid degradation according to CLP criteria: not rapidly degradable.

11.2 Bioaccumulation

Table 25: Summary of relevant information on bioaccumulation

Property	Description of key information	Value used for CSA / Discussion
Partition coefficient n-octanol/water (log value)	A partition coefficient study has been carried out according to OECD 107. The measured particient coefficient for DMDS is of 1.91 at 20°C.	**Value used for CSA:** Log Kow (Pow): 1.91 at 20 °C Based on DMDS Log Kow values traced in the public domain and which support result obtained in the frame of the OECD test, it can be considered that DMDS has a low potential for

Property	Description of key information	Value used for CSA / Discussion
		bioaccumulation according to the cut-off criterion (log Kow < 4) set out under CLP regulation.

11.2.1 Measured partition coefficient and bioaccumulation test data

Dimethyldisulfide octanol-water partition coefficient was determined according to OECD 107 guideline (shakeflask method). The study was GLP-compliant. The study was carried out at 20.6°C and pH 6.7 and all validity criteria were met. A log Kow value of 1.91 was found.

The data are relevant and adequate for classification purposes under CLP EC 1272/2008 regulation.

Data used for classification: log Kow = 1.91

11.2.2 Conclusion on bioaccumulation according to CLP criteria

According to regulation EC 1272/2008, an organic and non ionisable substance is considered to have a "real potential to bioconcentrate" if its log kow value is equal or superior to 4 (section 4.1.2.8.1 of CLP regulation EC 1272/2008). With its log Kow value of 1.91 and the absence of an experimental fish BCF, dimethyldisulfide is therefore not expected to have a real potential for bioaccumulation in animal tissues (log Kow < 4). **Dimethyldisulfide is therefore considered to have a low potential for bioaccumulation according to CLP criteria.**

11.3 Acute aquatic hazard

Table 26: Summary of relevant information on acute aquatic toxicity

Method	Results	Remarks	Reference
Oncorhynchus mykiss freshwater semi-static EPA OPPTS Draft Guideline 850.1075 (1996)	LC50 (96 h): 0.97 mg/L test mat. (meas. (geom. mean)) based on: mortality (95% CL: 0.96 - 0.98 mg/L)	1 (reliable without restriction) key study experimental result Test material (EC name): dimethyl disulphide	Scheerbaum D. (2007a)
Danio rerio freshwater semi-static EPA OPPTS Draft Guideline 850.1075 (1996)	LC50 (96 h): 5.01 mg/L test mat. (meas. (geom. mean)) based on: mortality (95% CL: 3.30 - 7.59 mg/L)	1 (reliable without restriction) supporting study experimental result Test material (EC name): dimethyl disulphide	Scheerbaum D. (2007b)
Cyprinodon variegatus freshwater	LC50 (96 h): 5.6 mg/L test mat. based on: mortality	1 (reliable without restriction) supporting study	Minderhout T., Kendall T.Z., Krueger H.O (2008a)

Method	**Results**	**Remarks**	**Reference**
semi-static EPA OPPTS 850.1075 (Freshwater and Saltwater Fish Acute Toxicity Test)		experimental result Test material (EC name): dimethyl disulphide	
Method	**Results**	Remarks	Reference
Daphnia magna *freshwater* *semi-static* *OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)*	EC50 (48 h): 1.82 mg/L test mat. (meas. (geom. mean)) based on: mobility (95% CL: 1.78 - 1.86 mg/L)	1 (reliable without restriction) key study experimental result Test material (EC name): dimethyl disulphide	Noack M (2007)
Daphnia magna *freshwater* *static* *OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)*	EC50 (48 h): 7 mg/L test mat. (meas. (not specified)) based on: mobility (95% confidence interval: 6.5 - 7.6 mg/L)	1 (reliable without restriction) supporting study experimental result Test material (EC name): dimethyl disulphide	Thiebaud H (1996)
Americamysis bahia *saltwater* *semi-static* *EPA OPPTS 850.1035 (Mysid Acute Toxicity Test)* *ASTM Standard E729-96: Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates and Amphibians*	LC50 (96 h): 5 mg/L test mat. (meas. (geom. mean)) based on: mortality (95% confidence interval: 2.5 to 10 mg a.i./L)	1 (reliable without restriction) key study experimental result Test material (EC name): dimethyl disulphide	Minderhout T., Kendall T.Z., Krueger H.O (2007a)
other aquatic mollusc: Eastern Oyster (Crassostrea virginica) *saltwater* *flow-through* *EPA OPPTS 850.1025 (Bivalve Acute Toxicity (shell deposition test))* *ASTM Standard E729-96: Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates and Amphibians*	EC50 (96 h): 14 mg/L test mat. (meas. (arithm. mean)) based on: mortality (95% confidence interval: 11 to 15 mg a.i./L)	1 (reliable without restriction) supporting study experimental result Test material (EC name): dimethyl disulphide	Minderhout T., Kendall T.Z., Krueger H.O (2007b)

Method	Results	Remarks	Reference
Method	Results	Remarks	Reference
Anabaena flos-aquae (algae) *freshwater* *static* *OECD Guideline 201 (Alga, Growth Inhibition Test)* *EPA OPPTS 850.5400 (Algal Toxicity, Tiers I and II)*	EC50 (96 h): 6.7 mg/L act. ingr. (meas. (arithm. mean)) based on: growth rate NOEC (96 h): 0.17 mg/L act. ingr. (meas. (arithm. mean)) based on: growth rate	1 (reliable without restriction) key study experimental result Test material (EC name): dimethyl disulphide	Minderhout T., Kendall T.Z., Krueger H.O (2008b)
Navicula pelliculosa (algae) *freshwater* *static* *OECD Guideline 201 (Alga, Growth Inhibition Test)* *EPA OPPTS 850.5400 (Algal Toxicity, Tiers I and II)*	EC50 (96 h): 25 mg/L act. ingr. (meas. (arithm. mean)) based on: growth rate NOEC (96 h): 15 mg/L act. ingr. (meas. (arithm. mean)) based on: growth rate	1 (reliable without restriction) supporting study experimental result Test material (EC name): dimethyl disulphide	Minderhout T., Kendall T.Z., Krueger H.O (2008c)
Pseudokirchnerella subcapitata (algae) *freshwater* *static* *OECD Guideline 201 (Alga, Growth Inhibition Test)* *EPA OPPTS 850.5400 (Algal Toxicity, Tiers I and II)*	EC50 (72 h): 25.6 mg/L test mat. (meas. (arithm. mean)) based on: growth rate (95% CL: 23.6 - 27.8 mg/L) NOEC (72 h): 9.4 mg/L test mat. (meas. (arithm. mean)) based on: growth rate	1 (reliable without restriction) supporting study experimental result Test material (EC name): dimethyl disulphide	Scheerbaum D. (2007c)
Pseudokirchnerella subcapitata (algae) *freshwater* *static* *OECD Guideline 201 (Alga, Growth Inhibition Test)*	EC50 (72 h): 35 mg/L test mat. (nominal) based on: growth rate NOEC (72 h): 10.43 mg/L test mat. (nominal) based on: growth rate EC10 (72 h): 9.3 mg/L test mat. (nominal) based on: growth rate	1 (reliable without restriction) supporting study experimental result Test material (EC name): dimethyl disulphide	Thiebaud H, Lespagnol CC (2000)
Skeletonema costatum (algae) *saltwater* *static* *OECD Guideline 201 (Alga, Growth Inhibition Test)* *EPA OPPTS 850.5400 (Algal Toxicity, Tiers I and II)*	EC50 (96 h): 3.9 mg/L act. ingr. (meas. (arithm. mean)) based on: growth rate NOEC (96 h): 0.95 mg/L act. ingr. (meas. (arithm. mean)) based on: growth rate	1 (reliable without restriction) key study experimental result Test material (EC name): dimethyl disulphide	Minderhout T., Kendall T.Z., Krueger H.O (2008d)

Method	Results	Remarks	Reference
Lemna gibba (aquatic plants) *freshwater* *semi-static* *OECD Guideline 221 (Lemna sp. Growth Inhibition test)* *EPA OPPTS 850.4400 (Aquatic Plant Toxicity Test using Lemna spp. Tiers I & II))*	EC50 (7 d): 31 mg/L act. ingr. (meas. (geom. mean)) based on: frond number (95% CL = 25 - 34 mg a.i./L) EC50 (7 d): 46 mg/L act. ingr. (meas. (geom. mean)) based on: biomass (95% CL = 41 - 50 mg/L) EC50 (7 d): 36 mg/L act. ingr. (meas. (geom. mean)) based on: frond number and growth rate (95% CL = 33 - 38 mg/L) EC50 (7 d): 75 mg/L act. ingr. (meas. (geom. mean)) based on: biomass and growth rate (95% CL = 67 - 84 mg/L)	1 (reliable without restriction) key study experimental result Test material (EC name): dimethyl disulphide	Minderhout T, Kendall TZ and Krueger HO (2008e)

11.3.1 Acute (short-term) toxicity to fish

Acute toxicity of dimethyldisulfide to fish has been assessed using different fish species according to international standards or accepted guidelines, equivalent to OECD 203 guideline. All studies were GLP compliant.

Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) has been investigated through EPA OPPTS Draft Guideline 850.1075 (1996). *Oncorhynchus mykiss* juveniles were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 0.625 - 1.25 - 2.5 - 5 and 10 mg/L under semi-static conditions. All validity criteria as set in the OECD 203 guideline were met. The results are based on geometric mean of concentrations measured in fresh and expired media. 96h-LC_{50} was 0.97 mg/L (geom. mean) and 96h-NOEC was 0.541 mg/L (geom. mean), (Scheerbaum, 2007a).

Acute toxicity to zebrafish (*Danio rerio*) has been investigated through EPA OPPTS Draft Guideline 850.1075 (1996). *Danio rerio* were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 6.25 - 12.5 - 25 - 50 and 100 mg/L under semi-static conditions. All validity criteria as set in the OECD 203 guideline were met. The results are based on geometric mean of concentrations measured in fresh and expired media. 96h-LC_{50} was 5.01 mg/L and 96h-NOEC was 3.3 mg/L (Scheerbaum, 2007b).

Acute toxicity to *Cyprinodon variegatus* has been investigated through EPA OPPTS 850.1075; U.S. Environmental Protection Agency Standard Evaluation Procedure for Acute Toxicity Test for Estuarine and Marine Organisms and ASTM Standard E729-96. *Cyprinodon variegatus* were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 2.6 - 4.3 - 7.2 - 12 and 20 mg/L under semi-static conditions. All validity criteria as set in the OECD 203 guideline were met. The results are based on geometric mean of concentrations measured in fresh and expired media. 96h-LC_{50} was 5.6 mg/L and 96h-NOEC was inferior to 2.3 mg/L (Minderhout, 2008a).

The data are relevant and adequate for classification purposes under CLP EC 1272/2008 regulation.

Data used for classification: fish 96h-LC_{50} = 0.97 mg/L (i.e., the lowest LC_{50} value obtained).

11.3.2 Acute (short-term) toxicity to aquatic invertebrates

Acute toxicity of dimethyldisulfide to invertebrates has been assessed using different freshwater and marine invertebrates species according to international standards or accepted guidelines. Tests on *Daphnia magna* were carried out according to guidelines equivalent to OECD 202 guideline. All studies were GLP compliant.

Freshwater species

Acute toxicity to *Daphnia magna* has been investigated through EPA OPPTS 850.1010 guideline. *Daphnia magna* were exposed for 48 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 0.970 - 2.13 - 4.70 - 10.3 - 22.7 and 50 mg/L under semi-static conditions. All validity criteria as set in the OECD 202 guideline were met. The results are based on geometric mean of concentrations measured in fresh and expired media. 48h-EC_{50} was 1.82 mg/L and 48h-NOEC was 0.618 mg/L (Noack, 2007).

In another test, *Daphnia magna* were exposed for 48 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 3.3 - 4.0 - 4.8 - 5.8 - 6.9 - 8.3 - 10.0 - 12.0 and 14.4 mg/L under static conditions. All validity criteria as set in the OECD 202 guideline were met. The results are based on nominal concentrations since measured concentrations in fresh and expired media were within the range of [80% – 120%] of nominal concentrations. 48h-EC_{50} was 7.0 mg/L (Thiebaud, 1996).

Three other EC_{50} data for *Daphnia spp*. immobilization are available: 48h-EC_{50} = 4 mg/L for *Daphnia magna* (Seppovaara 1970), 4h-EC_{50} = 21.4 mg/L for *Daphnia pulex* (Werner, 1963) and 24h-EC_{50} = 5 mg/L for *Daphnia sp*. (Dubreuil, 1984). These data are considered Klimisch 4e (i.e. little information available, documentation insufficient for assessment). Disregarding these studies is not critical for CLP classification as the results are far above the lowest 48h-EC_{50} recorded for invertebrates for dimethyldisulfide.

Marine species

Acute toxicity to Saltwater Mysid (*Americamysis bahia*) has been investigated through EPA OPPTS 850.1035, ASTM Standard E729-96 and U.S. Environmental Protection Agency, Standard Evaluation Procedure: Acute Toxicity Test for Estuarine and Marine Organisms. *Americamysis bahia* were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 1.1 - 2.3 - 4.5 - 9.0 and 18 mg/L under semi-static conditions. All validity criteria were met. The results are based on nominal concentrations since measured concentrations in fresh and expired media were within the range of [80% – 120%] of nominal concentrations. 96h-LC_{50} was 5 mg/L and 96h-NOEC was 2.5 mg/L (Minderhout, 2007a).

Acute toxicity has also been investigated for Eastern oyster under flow-through conditions. *Crassostrea virginica* were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 3.8 - 7.5 - 15 - 30 and 60 mg/L. All validity criteria were met. The results are based on arithmetic mean of concentrations measured at 0, 48 and 96 hours. 96h-EC_{50} was 14 mg/L and 96h-NOEC was 3 mg/L (Minderhout, 2007b).

The data are relevant and adequate for classification purposes under CLP EC 1272/2008 regulation.

Data used for classification: invertebrates 48h-EC_{50} = 1.82 mg/L (i.e., the lowest EC_{50} value obtained).

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

Acute toxicity of dimethyldisulfide to algae has been assessed using different freshwater and marine species according to international standards or accepted guidelines. All studies were GLP compliant.

Two studies were conducted according to OECD 201 guideline on *Pseudokirchneriella subcapitata*. In the first study, *Pseudokirchneriella subcapitata* cells were exposed for 72 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 5.3 - 9.5 - 17.2 - 30.8 - 55.6 and 100 mg/L under static conditions. All validity criteria were met. The results are based on initial measured concentrations since measured concentrations in fresh and expired media were within the range of [80% – 120%] of initial measured concentrations. For growth rate inhibition, 72h-ErC_{50} was 35 mg/L and 72h-ErC_{10} was 9.3 mg/L (Thiebaut, 2000). In the second study, *Pseudokirchneriella subcapitata* cells were exposed for 72 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 6.3 - 12.5 - 25 - 50 and 100 mg/L under static conditions. All validity criteria were met. The results are based on mean measured concentrations in fresh and expired media. For growth rate inhibition, 72h-ErC_{50} was 25.6 mg/L and 72h-NOEC was 9.4 mg/L (Scheerbaum, 2007c).

One study was conducted according to OECD 201 guideline on *Anabaena flosaquae*. *Anabaena flosaquae* cells were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 0.15 - 0.38 - 0.96 - 2.4 - 6.0 and 15 mg/L under static conditions. All validity criteria were met. The results are based on mean measured concentrations. For growth rate inhibition, 96h-ErC_{50} was 6.7 mg/L and 96h-NOEC was 0.17 mg/L (Minderhout, 2008b).

One study was conducted according to OECD 201 guideline on *Navicula pelliculosa*. *Navicula pelliculosa* cells were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 2.3 - 3.9 - 6.5 - 11.0 - 18.0 and 30 mg/L under static conditions. All validity criteria were met. The results are based on mean measured concentrations. For growth rate inhibition, 96h-ErC_{50} was 25 mg/L and 96h-NOEC was 15 mg/L (Minderhout, 2008c).

One study was conducted according to OECD 201 guideline on *Skeletonema costatum*. *Skeletonema costatum* cells were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 0.6 - 1.3 - 2.8 - 6.2 - 14.0 and 30.0 mg/L under static conditions. All validity criteria were met. The results are based on mean measured concentrations. For growth rate inhibition, 96h-ErC_{50} was 3.9 mg/L and 96h-NOEC was 0.95 mg/L (Minderhout, 2008d).

One study was conducted according to OECD 221 guideline on *Lemna gibba*. *Lemna gibba* were exposed for 7 days to an aqueous solution of dimethyldisulfide at different nominal concentrations of 3.1 - 6.3 - 13 - 25 - 50 and 100 mg a.i./L under semi-static conditions. All validity criteria were met. The results are based on mean measured concentrations. For frond number, 7d-EC_{50} was 36 mg/L and 7d-NOAEC was 5.5 mg/L (Minderhout, 2008e).

The data are relevant and adequate for classification purposes under CLP EC 1272/2008 regulation.

Data used for classification: algae 96h-ErC_{50} = 3.9 mg/L (i.e., the lowest ErC_{50} / EC_{50} value obtained).

11.4 Long-term aquatic hazard

Table 27: Summary of relevant information on chronic aquatic toxicity

Method	Results	Remarks	Reference
Pimephales promelas freshwater early-life stage: reproduction, (sub)lethal effects flow-through OECD Guideline 210 (Fish, Early-Life Stage Toxicity Test)	NOEC (33 d): 0.936 mg/L test mat. (meas. (geom. mean)) based on: fry survival LOEC (33 d): 1.87 mg/L test mat. (meas. (geom. mean)) based on: fry survival	1 (reliable without restriction) key study experimental result **Test material (common name): DMDS**	Rebstock, M. (2011)

Method	Results	Remarks	Reference
		Form: liquid	
Cyprinodon variegatus saltwater early-life stage: reproduction, (sub)lethal effects flow-through OECD Guideline 210 (Fish, Early-Life Stage Toxicity Test)	NOEC (38 d): 0.473 mg/L test mat. (meas. (geom. mean)) based on: hatchability and fry survival LOEC (38 d): 0.952 mg/L test mat. (meas. (geom. mean)) based on: hatchability and fry survival	1 (reliable without restriction) key study experimental result **Test material (common name): DMDS** Form: liquid	Gerke A. (2011a)

Method	Results	Remarks	Reference
Daphnia magna *freshwater* *semi-static* *OECD Guideline 211 (Daphnia magna Reproduction Test)*	NOEC (21 d): 0.0025 mg/L test mat. (nominal) based on: reproduction LOEC (21 d): 0.005 mg/L test mat. (nominal) based on: reproduction	1 (reliable without restriction) key study experimental result Test material (common name): DMDS Form: liquid	Rebstock M. (2011)
Americamysis bahia *saltwater* *flow-through* *EPA OPPTS 850.1350 (Mysid Chronic Toxicity Test)*	NOEC (28 d): 0.464 mg/L test mat. (meas. (geom. mean)) based on: mean total young per surviving pair LOEC (28 d): 0.9 mg/L test mat. (meas. (geom. mean)) based on: Mean total young per surviving pair	1 (reliable without restriction) key study experimental result Test material (common name): DMDS Form: liquid	Gerke A. (2011b)

11.4.1 Chronic toxicity to fish

The effect of the test item, Dimethyl disulphide (DMDS) on the fathead minnow (*Pimephales promelas*) embryos and fry during an early life-stage exposure was investigated under flow-through conditions, according to OECD 210 guideline. The test was conducted at nominal test concentrations of 0.13, 0.25, 0.50, 1.0, 2.0 and 4.0 mg/L. Due to loss of DMDS from volatility, analytical measured concentrations represented recoveries of 44 to 55% of nominal (mean measured concentrations were 0.0566, 0.122, 0.240, 0.549, 0.936 and 1.87 mg/L) and therefore the reported effect levels are based on mean measured concentrations.

Initiation of the study was to embryos <24 hours post fertilisation and sufficient embryos were used to give a group size of at least 15 embryos per replicate. There were 4 replicates for each treatment group. Day 0 post-hatch was based on ≥ 95% hatch in the control group. Day 0 was reached after 5 days incubation. All live fry were counted and released into their respective replicate growth chambers on day 7 post-hatch and exposure continued for 28 days post-hatch. Survival was monitored daily by visually inspecting each test chamber, and any behavioural or physical changes were recorded, including abnormalities. After 33 days exposure

(including the egg incubation period) the fish were euthanised and total length and blotted weight were measured.

Hatching success in the control and vehicle control was 98 and 100%, respectively, and ranged from 95 to 100% in the DMDS treatments. Post-hatch survival of fry in the control and vehicle control was 94 and 91%, respectively, and ranged from 75 to 94% in the DMDS treatments. Mean total length was 23.3 and 23.0 mm in the control and vehicle control, respectively, and ranged from 21.6 to 22.9 mm in the DMDS treatments. Mean blotted wet weight was 0.101 and 0.107 g in the control and vehicle control, respectively, and ranged from 0.101 to 0.113 g in the DMDS treatments. Water quality parameters were within acceptable limits throughout the exposure. All validity criteria were met.

Based on geometric mean measured concentrations of DMDS, the NOEC and LOEC values for *P. promelas* egg hatchability, total length, and blotted wet weight were 1.87 and >1.87 mg/L, respectively (i.e. no effect was seen on these endpoints in this study).

Based on geometric mean measured concentrations of DMDS, the NOEC was 0.94 mg/L for larval survival (Rebstock, 2011).

The effect of the test item, Dimethyl disulphide (DMDS) on the sheepshead minnow (*Cyprinodon variegatus*) embryos and fry during an early life-stage exposure was investigated under flow-through conditions. The test was conducted at nominal test concentrations of 0.065, 0.13, 0.25, 0.50, 1.0, and 2.0 mg/L. Due to loss of DMDS from volatility, analytical measured concentrations represented recoveries of 44 to 55% of nominal (mean measured concentrations were 0.0288, 0.0615, 0.123, 0.229, 0.473, and 0.952 mg/L) and therefore the reported effect levels are based on geometric mean measured concentrations.

Initiation of the study was to embryos <24 hours post fertilisation and sufficient embryos were used to give a group size of at least 15 embryos per replicate. There were 4 replicates for each treatment group. Day 0 post-hatch was based on ≥95% hatch in the control group. Day 0 was reached after 10 days incubation. All live fry were counted and released into their respective replicate growth chambers on day 7 post-hatch and exposure continued for 28 days post-hatch. Survival was monitored daily by visually inspecting each test chamber, and any behavioural or physical changes were recorded, including abnormalities. After 38 days exposure (including the egg incubation period) the fish were euthanasised and total length and blotted weight were measured.

Hatching success in the control and vehicle control was 94 and 93%, respectively, and ranged from 73 to 94% in the DMDS treatments. Post-hatch survival of fry in the control and vehicle control was 93 and 96%, respectively, and ranged from 50 to 99% in the DMDS treatments. Mean total length was 14.2 and 15.3 mm in the control and vehicle control, respectively, and ranged from 12.3 to 15.2 mm in the DMDS treatments. Mean blotted wet weight was 0.094 and 0.125 g in the control and vehicle control, respectively, and ranged from 0.072 to 0.124 g in the DMDS treatments. Water quality parameters were within acceptable limits throughout the exposure. All validity criteria were met.

Based on geometric mean measured concentrations of DMDS, the NOEC and LOEC values for fathead minnow egg hatchability, total length, and blotted wet weight were 1.87 and >1.87 mg a.i./L, respectively (i.e. no effect was seen on these endpoints in this study).

Based on mean measured concentrations of DMDS, the NOEC for larval survival was 0.47 mg/L (Gerke, 2011).

The data are relevant and adequate for classification purposes under CLP EC 1272/2008 regulation.

Data used for classification: fish 38d-NOEC = 0.47 mg/L (i.e., the lowest NOEC value obtained).

11.4.2 Chronic toxicity to aquatic invertebrates

A freshwater 21 day static-renewal chronic toxicity test with *Daphnia magna* was performed according to OECD Guideline 211 to assess the toxicity of dimethyldisulfide (DMDS) at nominal test concentrations of 0

(control), 0 (solvent control), 0.0013, 0.0025, 0.0050, 0.010, and 0.020 mg/L (concentrations selected on the basis of the results of a range finding test). The definitive test was conducted for 21 days commencing when daphnids (<24 hours old at the start of the test) were added to the test chambers. 10 replicates (1 daphnid per test container) were included and the daphnids were fed daily. Each day the parental generation daphnids were transferred to clean test chambers containing freshly prepared test solutions. Observations were made daily on the number of surviving adult daphnids, occurrence of abnormalities, and production of neonates. The neonates produced between each renewal and at termination were counted and discarded. At test termination, the length of each surviving adult was measured and the mean adult dry weight for the controls and each test substance treatment was determined after combining the surviving adult daphnids by treatment. Water quality measurements and water temperature were monitored throughout the test. The results are based on nominal concentrations of DMDS since the test concentrations (in both fresh and expired media) were outside the range of the analytical method, with the exception of the top test concentration. Statistical analysis was performed to assess the effects of DMDS on adult survival, reproduction and adult length.

There was no significant reduction in survival in any test treatment as compared to the dilution water control survival. Based on parent survival, the 21-day NOEC and LOEC were 0.020 and >0.020 mg/L, respectively (i.e., no effect was seen on this endpoint in this study). Regarding parental length at the end of the study, the 21-day NOEC was 0.01 mg/L. Based on the total numbers of live young produced per surviving parent daphnids, the 21-day NOEC was 0.0025 mg/L (Rebstock, 2011).

A life-cycle toxicity test under flowthrough conditions with the saltwater mysid, *Americamysis bahia*, exposed to dimethyldisulfide (DMDS) was performed according to OPPTS 850.1350. The purpose of this test was to assess test substance effects on survival, growth, and reproduction of mysids, and to determine a no-observed-effect concentration (NOEC). Comparisons between control and exposure treatments were made to determine the test substance concentrations at which statistically significant reductions in the measured parameters occurred. Consideration was also given to the biological significance of any statistically significant reductions that were detected.

Based on mean measured concentrations of dimethyldisulfide during the 28-day exposure, the F0 mysid survival LC50 was >1.73 mg/L, and the NOEC was 1.73 mg/L, the highest concentration tested (i.e., no effect was seen on this endpoint during the study). The NOEC for F0 male and female mysid length on day 14, and female mysid length on day 28 was 1.73 mg/L, the highest concentration tested (i.e., no effect was seen on these endpoints during the study). The NOEC for F0 male mysid length on day 28 was 0.9 mg/L. The NOEC for F0 mysid day of first brood and mean number of total young produced per female were 0.9 and 0.46 mg/L, respectively. The NOEC for F1 mysid survival on day 10 was 0.861 mg/L, the highest concentration tested (i.e., no effect was seen on this endpoint during the study). The NOEC for F1 male and female mysid length on day 10 was 0.861 mg/L (Gerke, 2011).

The data are relevant and adequate for classification purposes under CLP EC 1272/2008 regulation.

Data used for classification: invertebrates 21d-NOEC = 0.0025 mg/L (i.e., the lowest NOEC value obtained).

11.4.3 Chronic toxicity to algae or other aquatic plants

See section 11.3.3.

The data are relevant and adequate for classification purposes under CLP EC 1272/2008 regulation.

Data used for classification: algae 96h-NOEC = 0.17 mg/L (i.e., the lowest NOEC/ErC_{10} value obtained).

11.5 Comparison with the CLP criteria

11.5.1 Acute aquatic hazard

The relevant data for acute aquatic hazard classification purposes under CLP EC 1272/2008 regulation are as follows:

Fish 96h-LC_{50} = 0.97 mg/L (i.e., the lowest LC_{50} value obtained).

Invertebrates 48h-EC50 = 1.82 mg/L (i.e., the lowest EC_{50} value obtained).

Algae 96h-ErC_{50} = 3.9 mg/L (i.e., the lowest ErC_{50} value obtained).

According to Table 4.1.0(a) of Annex I of Regulation EC 1272/2008, a substance is considered to fall into category aquatic acute tox 1 if any species within any of the three trophic levels (fish, invertebrates and algae) tested in the framework of short-term toxicity tests shows a LC_{50}/EC_{50} lower or equal to 1 mg/L of substance. In addition, a M-factor of 10 or higher is considered for substances for which short-term toxicity tests shows LC_{50}/EC_{50} lower or equal to 0.1 mg/L. Regarding dimethyldisulfide, the most sensitive trophic level was fish with a 96h-LC_{50} of 0.97 mg/L for rainbow trout. DMDS is therefore considered as *very toxic to aquatic life* (H400, M=1) in the REACh dossier. **According to CLP criteria and based on the evidence provided, classification as Aquatic Acute Tox 1 (H400, M=1) is proposed.**

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

Bioaccumulation: since DMDS log Kow is inferior to 4 (CLP cut-off criterion) and no experimental BCF is available, DMDS is considered to have a low potential for bioaccumulation according to the criteria set out under CLP regulation EC 1272/2008.

Degradation: since DMDS is not readily biodegradable, not hydrolysable at environmental temperatures and pH and no evidence of significant degradation in sediment or soil is provided, DMDS is considered not rapidly degradable in the environment according to CLP criteria.

The relevant ecotoxicity data for long-term aquatic hazard classification purposes under CLP regulation EC 1272/2008 are as follows:

- Long-term fish 38d-NOEC = 0.47 mg/L (i.e., the lowest NOEC value obtained).
- Long-term invertebrates 21d-NOEC = 0.0025 mg/L (i.e., the lowest NOEC value obtained).
- Long-term algae 96h-NOEC = 0.17 mg/L (i.e., the lowest NOEC value obtained).

According to Table 4.1.0(b)(i) of Annex I of Regulation EC 1272/2008, a non-rapidly degradable substance is considered to fall into category aquatic chronic tox 1 if any species within any of the three trophic levels (fish, invertebrates and algae) tested in the framework of long-term toxicity tests shows a NOEC/EC_{10} lower or equal to 0.1 mg/L of substance. In addition, a M-factor of 10 is considered for non-rapidly degradable substances for which long-term toxicity tests shows NOEC/EC_{10} comprised between 0.001 and 0.01 mg/L. Since DMDS is not rapidly biodegradable and the lowest long-term NOEC is comprised between 0.001 mg/L and 0.01 mg/L, DMDS is considered as *very toxic to aquatic life with long lasting effects* (H410, M=10) in the REACh dossier. **According to CLP criteria and based on the evidence provided, classification as Aquatic Chronic Tox 1 (H410, M=10) is proposed.**

11.6 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

CLASSIFICATION

- Acute aquatic hazard classification: Aquatic Acute Tox 1, Hazard Statement H400: very toxic to aquatic life (M=1).

- Long-term aquatic hazard classification: Aquatic Chronic Tox 1, Hazard Statement H410: very toxic to aquatic life with long lasting effects (M=10).

LABELLING

- Hazard Statement H410: very toxic to aquatic life with long lasting effects

Pictogram: GHS09: environment

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Dimethyl disulfide is not currently listed in Annex VI of the CLP Regulation (EC) 1272/2008. The Dossier Submitter (DS's) proposes that it should be classified as Aquatic Acute 1 – H400 (M-factor of 1) based on a 96-h LC_{50} of 0.97 mg/L for fish, and Aquatic Chronic 1 – H410 (M-factor of 10) based on a 21-d NOEC of 0.0025 mg/L for *Daphnia* and lack of rapid degradation.

Degradation

The substance is stable to hydrolysis with a half-life at 25 °C of >1 year at pH 4, 7 and 9.

A GLP-compliant ready biodegradation test according to OECD TG 310 (CO_2 in sealed vessels (headspace test)) resulted in 53 % degradation after 28 days (based on carbon dioxide evolution). A non-GLP ready biodegradation test according to OECD TG 301D (closed bottle test) resulted in <10 % degradation after 28 days (based on DOC removal). The substance was not inhibitory to micro-organisms at the test concentration. These methods are appropriate for such a volatile substance (the vapour pressure is 30 hPa at 20 °C). Based on these results, dimethyl disulfide is not readily biodegradable.

An aerobic water-sediment simulation test (OECD TG 308) indicated that the substance rapidly dissipates from water to the atmosphere during a 7-h period, at a rate directly proportional to the flow of air through the test system with little transfer to sediment (<5 % applied radioactivity). No transformation products were reported. This test guideline is not appropriate for volatile substances. A soil simulation test similarly showed that aerobic transformation in soil may occur (forming carbon dioxide and methanesulfonic acid) but is not a major degradation pathway (between 12 and 43 % removal was observed over 59 – 120 d in four different soils, but this included evaporation as well as degradation).

In summary, dimethyl disulfide does not undergo rapid abiotic degradation (the hydrolysis half-life is > 1 year at 25 °C at relevant pH), is not readily biodegradable and showed no evidence of rapid mineralisation or primary transformation in simulation studies. The DS therefore considered it to be not rapidly degradable.

Bioaccumulation

The octanol-water partition coefficient (log K_{ow}) is 1.91 at 20.6 °C (shake flask method). No further information is available. This is below the CLP criterion for a bioaccumulative substance (log K_{ow} >4), so the DS considers that dimethyl disulfide does not have potential to bioaccumulate in aquatic organisms.

Aquatic toxicity

Aquatic toxicity data are available for all three trophic levels, and a summary of the relevant information is provided in the following table (the key endpoints used in hazard classification are highlighted in bold). All study results are expressed in terms of geometric mean measured concentrations, unless stated otherwise. 95 % confidence intervals have been included in the table, where relevant, to give an indication of the variability of the data (they are close to the classification cut-off values for the lowest acute fish result).

Table 1: *Summary of relevant information on aquatic toxicity*

Method	**Test organism**	**Endpoint**	**Toxicity values in mg a.s./L**	**Reference**
Short-term toxicity to fish				
US EPA OPPTS 850.1075 (draft) (semi-static)	*Oncorhynchus mykiss* (Rainbow Trout)	96-h LC_{50}	**0.97** (95 % CI: 0.96 – 0.98)	Anonymous, 2007a
US EPA OPPTS 850.1075 (draft) (semi-static)	*Danio rerio* (Zebrafish)	96-h LC_{50}	5.01 (95 % CI: 3.30 – 7.59)	Anonymous, 2007b
US EPA OPPTS 850.1075 (semi-static)	*Cyprinodon variegatus* (Sheepshead Minnow)	96-h LC_{50}	5.6 (nominal – closed bottles used with minimal head space) (95 % CI: not provided)	Anonymous, 2008a
Long-term toxicity to fish				
OECD TG 210 (flow-through)	*Pimephales promelas* (Fathead Minnow)	33-d NOEC	0.936	Anonymous, 2011
OECD TG 210 (flow-through)	*Cyprinodon variegatus* (Sheepshead Minnow)	38-d NOEC	0.473	Anonymous, 2011a
Short-term toxicity to aquatic invertebrates[a]				
OECD TG 202 (semi-static)	*Daphnia magna*	48-h EC_{50}	1.82 (95 % CI: 1.78 – 1.86)	Noack, 2007
OECD TG 202 (static)	*Daphnia magna*	48-h EC_{50}	7 (measured, not specified) (95 % CI: 6.5 – 7.6)	Thiebaud, 1996
US EPA OPPTS 850.1035 (semi-static)	*Americamysis bahia* (mysid shrimp)	96-h LC_{50}	5 (95 % CI: 2.5 – 10)	Minderhout et al., 2007a
US EPA OPPTS 850.1025 (flow-through)	*Crassostrea virginica* (Eastern Oyster)	96-h EC_{50}	14 (95 % CI: 11 – 15)	Minderhout et al., 2007b
Long-term toxicity to aquatic invertebrates				
OECD TG 211 (semi-static)	*Daphnia magna*	21-d $NOEC_{repro}$	**0.0025** (nominal)	Rebstock, 2011
US EPA OPPTS 850.1350 (flow-through)	*Americamysis bahia* (mysid shrimp)	28-d $NOEC_{repro}$	0.464	Gerke, 2011b
Toxicity to algae and aquatic macrophytes[b]				
OECD TG 201 (static)	*Anabaena flos-aquae*	96-h E_rC_{50} 96-h NOE_rC	6.7 0.17 (based on arithmetic mean concentrations)	Minderhout et al., 2007a
OECD TG 201 (static)	*Navicula pelliculosa*	96-h E_rC_{50} 96-h NOE_rC	25 15 (based on arithmetic mean concentrations)	Minderhout et al., 2007b
OECD TG 201 (static)	*Pseudokirchneriella subcapitata*	72-h E_rC_{50} 72-h NOE_rC	25.6 (95 % CI: 23.6 – 27.8) 9.4 (based on arithmetic	Scheerbaum, 2007c

			mean concentrations)	
OECD TG 201 (static)	*Pseudokirchneriella subcapitata*	72-h E_rC_{50} 72-h E_rC_{10}	35 9.3 (based on nominal concentrations)	Thiebaud & Lespagnol, 2000
OECD TG 201 (static)	*Skeletonema costatum*	96-h E_rC_{50} 96-h NOE_rC	3.9 0.95 (based on arithmetic mean concentrations)	Minderhout et al., 2007d
OECD TG 221 (semi-static)	*Lemna gibba*	7-d E_rC_{50} 7-d NOE_rC	36 (95 % CI: 33 – 38) 5.5	Minderhout et al., 2007e

Note: a – Three further acute invertebrate studies are available but their validity is unassignable so they are not included here. None indicate a more sensitive end point value than the *O. mykiss* result.
b – 72-h results are provided in the section on public comments.
CI – confidence interval
Bold results are behind the dossier submitter's proposal

Comments received during public consultation

Four Member State Competent Authorities (MSCA) provided public comments. One agreed with the proposed classification with no further comment. One MSCA agreed with the proposed classification, but pointed out that the ongoing evaluation of the application to use the substance as a Plant Protection Product (PPP) may result in a different interpretation of some existing studies. For example, they indicated that the OECD TG 308 study is not appropriate for volatile substances, highlighted information on atmospheric degradation (which is not directly relevant to classification based on aquatic data) and also provided additional information for some algal toxicity end points, as follows:

- *Anabaena flos-aquae* (Minderhout et al., 2008b): 72-h E_rC_{50} = 5.10 mg/L; 72-h NOE_rC = 1.90 mg/L (mean measured).
- *Navicula pelliculosa* (Minderhout et al., 2008c): 72-h E_rC_{50} = 20.0 mg/L; 72-h NOE_rC = 9.5 mg/L (mean measured).
- *Skeletonema costatum* (Minderhout et al., 2008d): 72-h E_rC_{50} = 3.6 mg/L; 72-h NOE_rC = 2.6 mg/L (mean measured).

None of these values affects the proposal.

Two other MSCAs asked whether further information could be provided about the likely substance concentration at the level of the NOEC in the most sensitive long-term *Daphnia magna* study (which was based on nominal concentrations only). In response, the Dossier Submitter stated that concentrations were reliably measured in the two highest treatments (0.01 and 0.02 mg/L), and losses were ≤40 % (except on one occasion when the concentration was below the method quantification limit, which may be related to an analytical or technical error). The extent of losses in the other treatments is unknown. Losses above 60 % at the level of the NOEC would result in a lower M-factor, but no conclusion can be drawn.

One of these MSCAs also highlighted an additional long-term *Daphnia magna* toxicity study from Japan, which gave a 21-d NOEC of 0.089 mg/L. In response, the Dossier Submitter provided further details and considers the study to be valid. The NOEC is based on time-weighted mean concentrations. The study used a semi-static exposure system with daily renewal, and chemical analysis indicated that losses were higher than 20 % (the maximum loss was 54 % on one occasion for one treatment). These losses are

comparable to the losses observed for the two highest treatments in the Rebstock study (2011). However, it is not appropriate to extrapolate to lower concentrations.

Assessment and comparison with the classification criteria

Degradation

Dimethyl disulfide does not undergo rapid abiotic degradation under relevant environmental conditions (the hydrolysis half-life is > 1 year at pH 4, 7 and 9 at 25 °C) and is not readily biodegradable. Simulation data do not provide evidence of rapid mineralisation. It is therefore not considered to be rapidly degradable according to the CLP Regulation.

Bioaccumulation

The substance is not potentially bioaccumulative because it has a log K_{ow} value (1.91) below the CLP Regulation threshold of 4.

Aquatic toxicity

Acute

Short-term aquatic toxicity data are available for three trophic levels. The substance is volatile, so will evaporate from test solutions if suitable precautions are not taken. The key acute study result for fish (Anonymous, 2007a) is based on mean measured concentrations from a semi-static test, so is suitably precautionary (it might over-estimate toxicity). The key chronic study for invertebrates (Rebstock, 2011) also used semi-static conditions, but the result is expressed in terms of nominal concentrations only. It is therefore likely that actual concentrations were lower (see public consultation comments).

The lowest acute toxicity value is a 96-h LC_{50} of 0.97 mg/L for *Oncorhynchus mykiss*. As this is below 1 mg/L, the substance meets the criteria for classification with **Aquatic Acute 1; H400 with an M-factor of 1**.

Chronic

Reliable long-term aquatic toxicity data are available for three trophic levels. There are no long-term toxicity data for the most sensitive fish species in acute tests (*O. mykiss*). This is not discussed in the CLH dossier. If the acute:chronic ratio for *C. variegatus* (11.8) is applied, an equivalent long-term NOEC for *O. mykiss* may be around 0.08 mg/L. This is less sensitive than the lowest long-term invertebrate NOEC by an order of magnitude so makes no difference to the classification.

The lowest long-term toxicity value is a 21-d NOEC of 0.0025 mg/L for *Daphnia magna*. As this is below 0.1 mg/L and the substance is not rapidly degradable, it meets the criteria for classification with **Aquatic Chronic 1; H410 with an M-factor of 10.**

In summary, RAC supports the Dossier Submitter's proposal.

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Testing laboratory: Products Safety Labs, 725 Cranbury Road, East Brunswick, NJ 08816, USA., Report no: T-5151. Study number: 23224, Report date: Aug 30, 1985	assay
Skorczynski S 2007: Anaerobic soil metabolism of dimethyl disulfide (study report), Testing laboratory: Cerexagri, Inc., 900 First Avenue, King of Prussia, PA 19406, USA, Report no: KP-2006-24. Owner company; Arkema, Study number: 31239, Report date: Jan 25, 2007	According to the Guidance on the application of the CLP criteria (v4.0, Nov 2013, p. 586), "*data regarding anaerobic degradation cannot be used in relation to deciding whether a substance should regarded as rapidly degradable*".
Tansy MF, Kendall FM, Fantasia J, landin WE and Oberly R 1981: Acute and Subchronic Toxicity studies of Rats Exposed to Vapors of Methyl Mercaptan and Other Reduced-Sulfur Compounds (publication), Toxic. Environm. Health, 8, 71-88. Study number: 6278, Report date:	Disregarded study due to major methodological deficiencies
Völkel 2006: Adsorption/desorption of DMDS on soils (study report), Testing laboratory: RCC Ltd., Environmental Chemistry & Pharmanalytics, CH-4452 Itingen, Switzerland, Report no: 856203. Owner company; Arkema, Report date:	Study not relevant for classification purposes with regards to hazard classes set under CLP regulation EC 1272/2008.
Völkel W 2006: DMDS: Adsorption-desorption of DMDS on one soil (study report), Testing laboratory: RCC Ltd., Environmental Chemistry & Pharmanalytics, CH-4452 Itingen, Switzerland, Report no: 858327. Owner company; Arkema, Study number: 33425, Report date: Jun 19, 2006	Study not relevant for classification purposes with regards to hazard classes set under CLP regulation EC 1272/2008.
YIN, F. et al, 1990.J. Atm. Chem., 11, 309-64. : No information Report date:	Study not relevant for classification purposes with regards to hazard classes set under CLP regulation EC 1272/2008.
YIN, F. et al, 1990.J. Atm. Chem., 11, 365-99. : No information Report date:	Study not relevant for classification purposes with regards to hazard classes set under CLP regulation EC 1272/2008.

13 ANNEXE I

Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Dimethyl disulphide

EC Number: **210-871-0**

CAS Number: **624-92-0**

Index Number: **/**

Contact details for dossier submitter: **ARKEMA France**

Version number: 1.4 **Date: March 2017**

CONTENTS

1 IDENTITY OF THE SUBSTANCE 1
1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE 1
1.2 COMPOSITION OF THE SUBSTANCE 1
2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING 3
2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA 3
3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING 5
4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL 5
5 IDENTIFIED USES 6
6 DATA SOURCES 7
7 PHYSICOCHEMICAL PROPERTIES 9
8 EVALUATION OF PHYSICAL HAZARDS 10
8.1 FLAMMABLE LIQUIDS 10
8.1.1 Short summary and overall relevance of the provided information on flammable liquids 10
8.1.2 Comparison with the CLP criteria 10
8.1.3 Conclusion on classification and labelling for flammable liquids 10
9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 10
9.1 SHORT SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED TOXICOKINETIC INFORMATION ON THE PROPOSED CLASSIFICATION(S) 11
10 EVALUATION OF HEALTH HAZARDS 14
10.1 ACUTE TOXICITY - ORAL ROUTE 14
10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity 16
10.1.2 Comparison with the CLP criteria 16
10.1.3 Conclusion on classification and labelling for acute oral toxicity 16
10.2 ACUTE TOXICITY - DERMAL ROUTE 17
10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity 18
10.2.2 Comparison with the CLP criteria 18
10.2.3 Conclusion on classification and labelling for acute dermal toxicity 18
10.3 ACUTE TOXICITY - INHALATION ROUTE 18
10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity 18
10.3.2 Comparison with the CLP criteria 19
10.3.3 Conclusion on classification and labelling for acute inhalation toxicity 19
10.4 SKIN CORROSION/IRRITATION 22
10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation 23
10.4.2 Comparison with the CLP criteria 23
10.4.3 Conclusion on classification and labelling for skin corrosion/irritation 23
10.5 SERIOUS EYE DAMAGE/EYE IRRITATION 24
10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation 26
10.5.2 Comparison with the CLP criteria 26
10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation 26
10.6 RESPIRATORY SENSITISATION 28
10.7 SKIN SENSITISATION 28
10.7.1 Short summary and overall relevance of the provided information on skin sensitisation 29
10.7.2 Comparison with the CLP criteria 32

10.7.3 Conclusion on classification and labelling for skin sensitisation ... *32*
10.8 GERM CELL MUTAGENICITY ... 36
10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity ... *40*
10.8.2 Comparison with the CLP criteria ... *41*
10.8.3 Conclusion on classification and labelling for germ cell mutagenicity ... *42*
10.9 CARCINOGENICITY ... 44
10.10 REPRODUCTIVE TOXICITY ... 44
10.10.1 Adverse effects on sexual function and fertility ... *44*
10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility ... *46*
10.10.3 Comparison with the CLP criteria ... *47*
10.10.4 Adverse effects on development ... *47*
10.10.5 Short summary and overall relevance of the provided information on adverse effects on development *48*
10.10.6 Comparison with the CLP criteria ... *49*
10.10.7 Adverse effects on or via lactation ... *49*
10.10.8 Conclusion on classification and labelling for reproductive toxicity ... *49*
10.11 SPECIFIC TARGET ORGAN TOXICITY-SINGLE EXPOSURE ... 75
10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure ... *75*
10.11.2 Comparison with the CLP criteria ... *77*
10.11.3 Conclusion on classification and labelling for STOT SE ... *77*
10.12 SPECIFIC TARGET ORGAN TOXICITY-REPEATED EXPOSURE ... 82
10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure ... *84*
10.12.2 Comparison with the CLP criteria ... *85*
10.12.3 Conclusion on classification and labelling for STOT RE ... *85*
10.13 ASPIRATION HAZARD ... 91

11 EVALUATION OF ENVIRONMENTAL HAZARDS ... 91

11.1 RAPID DEGRADABILITY OF ORGANIC SUBSTANCES ... 91
11.1.1 Ready biodegradability ... *93*
11.1.2 Degradation simulation testing in sediment (aerobic) ... *93*
11.1.3 Degradation simulation testing in soil (aerobic) ... *93*
11.1.4 Hydrolysis ... *93*
11.1.5 Conclusion on rapid degradability according to CLP criteria ... *94*
11.2 BIOACCUMULATION ... 94
11.2.1 Measured partition coefficient and bioaccumulation test data ... *95*
11.2.2 Conclusion on bioaccumulation according to CLP criteria ... *95*
11.3 ACUTE AQUATIC HAZARD ... 95
11.3.1 Acute (short-term) toxicity to fish ... *98*
11.3.2 Acute (short-term) toxicity to aquatic invertebrates ... *99*
11.3.3 Acute (short-term) toxicity to algae or other aquatic plants ... *99*
11.4 LONG-TERM AQUATIC HAZARD ... 100
11.4.1 Chronic toxicity to fish ... *101*
11.4.2 Chronic toxicity to aquatic invertebrates ... *102*
11.4.3 Chronic toxicity to algae or other aquatic plants ... *103*
11.5 COMPARISON WITH THE CLP CRITERIA ... 104
11.5.1 Acute aquatic hazard ... *104*
11.5.2 Long-term aquatic hazard (including bioaccumulation potential and degradation) ... *104*
11.6 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS ... 104

12 REFERENCES ... 109

12.1 REFERENCES QUOTED IN THE REGISTRATION DOSSIER AND THE CHL REPORT ... 109
12.2 REFERENCES QUOTED IN THE REGISTRATION DOSSIER AND NOT QUOTED IN THE CHL REPORT ... 115

13 ANNEXE I ... 119

1 PHYSICAL HAZARDS ... 5

1.1 FLAMMABLE LIQUID ... 5

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 6

3 HEALTH HAZARDS 19

3.1 ACUTE TOXICITY - ORAL ROUTE 19
3.2 ACUTE TOXICITY - DERMAL ROUTE 32
3.3 ACUTE TOXICITY - INHALATION ROUTE 43
3.4 ACUTE TOXICITY – OTHER ROUTES 69
3.5 SKIN CORROSION/IRRITATION 69
3.6 SERIOUS EYE DAMAGE/EYE IRRITATION 76
3.7 SKIN SENSITISATION 84
3.8 REPEATED DOSE TOXICITY – INHALATION ROUTE 95
3.9 REPEATED DOSE TOXICITY – DERMAL ROUTE 117
3.10 GERM CELL MUTAGENICITY 121
3.10.1 In vitro data 121
3.10.2 In vivo 140
3.11 REPRODUCTIVE TOXICITY 153
3.11.1 Toxicity to reproduction 153
3.11.2 Development toxicity – Teratogenicity 180
3.11.3 Other data 197
3.12 NEUROTOXICITY 199
3.13 IMMUNOTOXICITY 209

4 ENVIRONMENTAL HAZARDS 216

4.1 STABILITY 216
4.1.1 Hydrolysis 216
4.2 BIODEGRADATION 219
4.2.1 Biodegradation in water: screening tests 219
4.2.2 Degradation simulation testing in sediment 223
4.3 BIOACCUMULATION 227
4.3.1 Bioaccumulation: aquatic / sediment 227
4.4 ACUTE TOXICITY 228
4.4.1 Short-term toxicity to fish 228
4.4.2 Short-term toxicity to aquatic invertebrates 236
4.4.3 Algal growth inhibition tests 249
4.5 CHRONIC TOXICITY 263
4.5.1 Fish early-life stage (FELS) toxicity test 263
4.5.2 Chronic toxicity to aquatic invertebrates 269

1 PHYSICAL HAZARDS

1.1 Flammable liquid

[Study 1] Mak / K1 KS/ Flash point

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title	Bibliographic source
study report	Key study	1 (reliable with restrictions)	Mak WA	2005	Flammability of dimethyldisulfide TC	TNO Defense Security and safety , Rijswik, the Netherlands

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	EU Method A.9 (Flash-Point)	

Type of method : closed cup

GLP compliance : yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Any other information on materials and methods incl. tables

The flaspoint of the test substance was determined in conformity with the test method described in ISO 3679 and in accordance with test Guideline A.9 of the EC Directive 92/69/EEC. The setaflash closed cup flammability tester was used.the substance is placed in a test vessel, which is progressively heated until the vapour reaches a sufficiently high concentration in air to produce flammable miwture, which can be ignited.

Results and discussions

Flash point : 15 °C

at 101.282 kPa

[Study 2] Dean Handbook of organic chemistry/ K2/Flash point

Study reference

Reference type	Purpose Flag	Reliability	Author	Year	Title	Bibliographic source
review article or handbook		2 (reliable with restrictions)	Dean JA	1987	Handbook of organic chemistry	Handbook of organic chemistry, New York, NY: McGraw-hill book Co. 1987 p1-208

Materials and methods

Type of method : closed cup

GLP compliance : no data

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Results and discussions

Flash point : 24 °C

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

[Study 1] Susman, 1978/K2 SS/Basic toxicokinetics

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**	**Bibliographic source**
publication		2 (reliable with restrictions)	Susman JL, Hornig JF, Thomae, SC, Smith RP	1978	Pulmonary excretion of hydrogen sulfide, methanethiol, dimethyl sulfide and dimethyl disulfide in mice	Drug Chem. Toxicol., 1(4), 327-338.

Materials and methods

Type of method : in vivo

Objective of study: metabolism

Test guideline

Qualifier	**Guideline**	**Deviations**
no guideline followed		

GLP compliance : no

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Test animals

Species : mouse

Strain : CD-1

Sex : female

Details on test animals and environmental conditions
TEST ORGANISMS:
- Source: Charles River Laboratories, Wilmington, MA, USA
- Age: no data- Weight at study initiation: 25-30 g
- Adaptation period: no data
HOUSING
The animals were housed 5 per cages
FOOD and WATER
- Food: ad libitum
- Water: ad libitum
ENVIRONMENTAL CONDITIONS
- Temperature : no data
- Relative humidity : no data
- Light/dark cycle : no data
- Ventilation : no data

<u>Administration / exposure</u>

Route of administration :intraperitoneal

Vehicle : unchanged (no vehicle)

Duration and frequency of treatment / exposure : Single administration

Doses / concentrations : 1 µl/mouse

No. of animals per sex per dose : 5

Control animals : yes, concurrent no treatment

Positive control : not appropriate

<u>Results and discussions</u>

Pharmacokinetic studies

Details on excretion
Intraperitoneal administration of 1 µl DMDS/mouse resulted in its appearance in the expired air, as well as much smaller amounts of methyl mercaptan and dimethyl sulfide. The excretion of DMDS reached a peak between 3 and 6 minutes after i.p. injection, and the total amount eventually excreted was about 6% of the dose. The amount of dimethyl sulfide and methanethiol excreted accounted for about 0.5% of the injected dose.

Metabolite characterisation studies

Metabolites identified :yes

Details on metabolites : Methyl mercaptan and dimethyl sulfide

Bioaccessibility

Any other information on results incl. tables
Mice injected with 1 microliter of dimethyl disulfide (specific gravity 1.06 gram/ml at 20° C) excreted three volatile sulfur compounds in their expired breath. These were positively identified by gas chromatography/mass spectrometry as dimethyl disulfide, dimethyl sulfide and methanethiol. The pattern of the pulmonary excretion of these campounds with time was then followed using the gas chromatograph, and the results are summarized in the Table. The excretion of dimethyl disulfide reached a peak between 3 and 6 minutes after intraperitoneal injection, and the total amount eventually excreted was about 6%, of the dose. The amounts of dimethyl sulfide and methanethiol excreted accounted for about 0.5% each of the injected dose, and the peak excretion for each occurred at 6 minutes after injection. These results strongly suggest that dimethyl disulfide is split in vivo to yield two moles of methanethiol. Part of the latter is presurcably methylated to yield dimethyl sulfide.
This postulated sequence was proved by injecting methanethiol into one mouse and showing that both it and dimethyl

sulfide appeared on the expired breath. When dimethyl sulfide was injected into another mouse, it alone appeared In the expired breath.

Pulmonary Excretion of Volatile sulfur Compounds in Mice Following Intraperitoneal Injection of Dimethyl Disulfide[a]

Minutes afterInjection	Amount in Micrograms[b]		
	Dimethyl Disulfide	Dimethyl Sulfide	Methanethiol
0[c]	0.023 ± 0.013	0.001	0.001
3	2.1 ± 0.3	0.005 ± 0.0008	0.003 ± 0.0005
6	2.0 ± 0.14	0.14 ± 0.034	0.1 ± 0.016
9	0.4 ± 0.14	0.036 ± 0.0053	0.002 ± 0.0002
15	0.28 ± 0.068	0.016 ± 0.0037	0.001
22	0.04 ± 0.02	0.001	0.001
27	0.008	0.001	0.001
30	0.001	0.001	0.001
40	0.001	0.001	0.001

[a]Dose of 1 microliter/mouse or 35 to 40 mg/kg.
[b]Mean ± SEM for five mice. Amounts shown represent collections for 15 sec only. The total amounts were estimated by planimetry under the derived curves of above amounts vs. time.
[c]Values immediately after injection. No endogenous sulfur compounds were found prior to treatment.

<u>**Applicant's summary and conclusion**</u>

Conclusions

The intraperitoneal administration of 1 µl of dimethyl disulfide to mice resulted in its appearance in the expired breath as well as much smaller amounts of both methanethiol and dimethyl sulfide.

[Study 2] RA DPDS/Germain, 2008/K2 SS/Basic toxicokinetics

<u>**Study Reference**</u>

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**	**Bibliographic source**
publication		2 (reliable with restrictions)	GERMAIN E, SEMON E, SIESS M-H, TEYSSIER C	2008	Disposition and metabolism of dipropyl disulphide in vivo in rat	Xenobiotica, 38(1), 87-97

<u>**Materials and methods**</u>

Type of method : in vivo

Test guideline

Qualifier	**Guideline**	**Deviations**
no guideline followed		

GLP compliance : no data

<u>**Test materials**</u>

Identity of test material same as for substance defined in section 1 (if not read-across) : no

Test material identity

Identifier	Identity
common name	di-n-propyl disulphide
CAS number	629-19-6
EC number	211-079-8
IUPAC name	1-propyldisulfanylpropane

Radiolabelling : no

Details on test material
- Molecular formula : C6 H14 S2
- Molecular weight : 150.31
- Smiles notation : CCCSSCCC
- InChl : InChI=1/C6H14S2/c1-3-5-7-8-6-4-2/h3-6H2,1-2H3
- Structural formula : CH3CH2CH2SSCH2CH2CH3
- Physical state: liquid
- Analytical purity: 98%

Test animals

Species : rat

Strain :
Wistar

Sex : male

Details on test animals and environmental conditions
TEST ANIMALS
- Source: Janvier, Le Genest, Saint Isle, France
- Age at study initiation: 6-week-old
- Weight at study initiation: 26
- Fasting period before study: 18 h before and 5 h after treatment
- Housing: individually in stainless wire cages
- Individual metabolism cages: yes
- Diet : ad libitum with a semi-synthetic diet consisting of (on a dry matter basis) 18% casein, 43% starch, 21% saccharose, 5% corn oil, 2% cellulose, 5% mineral mixture, and 1% vitamin mixture for 1 week before the beginning of the experiment. Water was added to the diet in the ratio 50g water/100 g dry matter. The liquid diet was prepared daily
- Water : ad libitum
- Acclimation period: no data

ENVIRONMENTAL CONDITIONS
- Temperature (°C): no data
- Humidity (%): no data
- Air changes (per hr): no data
- Photoperiod (hrs dark / hrs light): no data

Administration / exposure

Route of administration : oral: gavage

Vehicle : corn oil

Duration and frequency of treatment / exposure : single administration

Doses / concentrations : 200 mg/kg

No. of animals per sex per dose : 3

Control animals : yes, concurrent vehicle

Details on study design

DPDS, dissolved in corn oil, was administered by gastric intubation at a dose of 200 mg/kg body weight. This dose was selected because it is equivalent to dose consumed per rat and per day in previous experiments showing an inhibiting effect of DPDS on the genotoxicity of carcinogens (Guyonnet et al. 2001). Three animals were included as negative controls in order to check that no sulphur compounds were detectable in their tissues. After oral administration, animals were maintained separately in metabolic cages. In each experiment, three animals were killed by exsanguination under anaesthesia (isoflurane 2.5% in oxygen) at 1, 2, 4, 8, 24, 36, and 48 h after oral dosing. Blood samples were withdrawn into tubes containing EDTA. Livers, stomachs, and intestines (40 cm long from the stomach) were removed, weighed, and stored at -20°C for a few days until further analysis. Blood was immediately centrifuged and plasma was stored at -20°C for a few days before analysis. Stomachs, intestines and livers were homogenized in distilled water. p-Cumene in ethanol solution (0.17mg/ml) was added as an internal standard. After trichloroacetic acid protein precipitation, the homogenate was centrifuged and the supernatant extracted three rimes with dichloromethane. The same protocol without homogenization was used for the plasma samples. The samples were then concentrated by evaporation under a nitrogen stream to a final volume of 500 µl. To this volume, 100 µl of nonane dissolved in dichloromethane (0.147mg/ml) was added in order to standardize the sample concentration and an aliquot of 1µl was injected into the gas chromatograph.

Statistics

Not appropriate

<u>**Results and discussions**</u>

Pharmacokinetic studies

Details on distribution in tissues

Identification of the detected products:When DPDS was administered to rats, this molecule was detected by GC-MS in all extracted tissues (stomach, intestine, liver and plasma). Propyl mercaptan (PM), methyl propyl mercaptan (MPS), methyl propyl sulfoxide (MPSO), and methyl propyl sulfone (MPSO2) were additional metabolites and were identified by comparing their retention tunes and fragmentation spectra with those of standards and/or those derived from a spectra library (Wiley library). The attached document (Figure 3) presents, for each organ, die quantities of these sulphur compounds as a function of time.Profile of DPDS in the organs examined:One hour after administration, DPDS was mainly detected in the stomach (Figure 3A). The absorption of DPDS is probably almost complete in this organ, as the DPDS quantity in the intestine is quite negligible (less than 0.01 µmol in the intestine compared with more than 50 µmol in the stomach at 2h). For these two organs, the amount of DPDS declined rapidly between 4 and 8h after administration.Profile of DPDS metabolites in the organs examined:In each organ, the metabolites were detected with various rates of appearance and different concentration-time profiles. In the stomach, DPDS and PM were detected very early (from the first hour after administration). The DPDS concentration-time profile was similar to that of PM. However, the concentrations of DPDS were higher than the concentrations of PM (Figure 3A). In the Iiver, MPS appeared first and transiently and then followed by the appearance of MPSO, and MPSO2. Whereas PM, MPS and MPSO were detected 1h after administration of DPDS, MPSO2 appeared only at 2h and its concentration gradually increased until 24h and then gradually decreased. At 48h its concentration was comparable to that observed at 8 h. The slow appearance of MPSO2 in the liver suggested a slow biotransformation from its precursor MPSO. MPSO2 also demonstrated a transient high concentration in the liver. In plasma (Figure 3D), PM reached a maximal concentration very early whereas the maximal concentration of MPSO was at 8h, and that of MPSO2 was delayed to 24h. In the stomach and in the intestine, the maximal concentrations were at 8h for MPSO and at 24h for MPSO2. The presence of these metabolites in the digestive tract at this time was probably caused by the introduction of these compounds at this time rather than an in situ metabolic transformation of DPDS or PM. The quantity of MPSO2 in stomach and intestine represented 7.9 and 9.6%, respectively, of that of the liver.

Bioaccessibility

Any other information on results incl. tables

The plasma concentration-time curves of DPDS and its metabolites after oral administration to rats are shown in Figure 4 (attached document) and the pharmacokinetic parameters are summarized in following Table. All parameters were determined only for DPDS as the plasmatic clearance could only be determined for the administered compound. The t1/2 (between 2 and 8 h) were quite similar for all metabolites, except MPSO2, whose t1/2 was 30 h. The

bioavailability, measured by the AUC of each compound, was very low for DPDS (0.008 h mM), because it was immediately transformed after administration. The AUC values were higher for MPSO and MPSO2 with values of 9.64 and 24.15 h mM, respectively. The Tmax values for the majority of these sulphur compounds were short (between 4 and 8h) indicating the non-persistence of these compounds in the plasma, whereas the Tm., for MPSO2 was delayed to 24h.

Pharmacokinetics parameters of dipropyl disulphide (DPDS) and its metabolites after a single oral administration of DPDS in the rat.

	Dipropyl disulphide (DPDS)	Propyl mercaptan (PM)	Methylpropyl sulphide (MPS)	Methylpropyl sulphoxide (MPSO)	Methylpropyl sulphone ($MPSO_2$)
$t_{1/2}$(h)	8.25	2.08	6.30	5.85	29.57
C_{max}(mM)	0.001	0.263	0.145	0.670	0.419
t_{max}(h)	4	8	8	8	24
AUC_{total}(h mM)	0.008	3.44	2.07	9.64	24.15
Cl_p($l.h^{-1}$)	39.57	n.c.	n.c.	n.c.	n.c.

n.c., not calculated.
Parameters were calculated from three rats.

Overall remarks, attachments

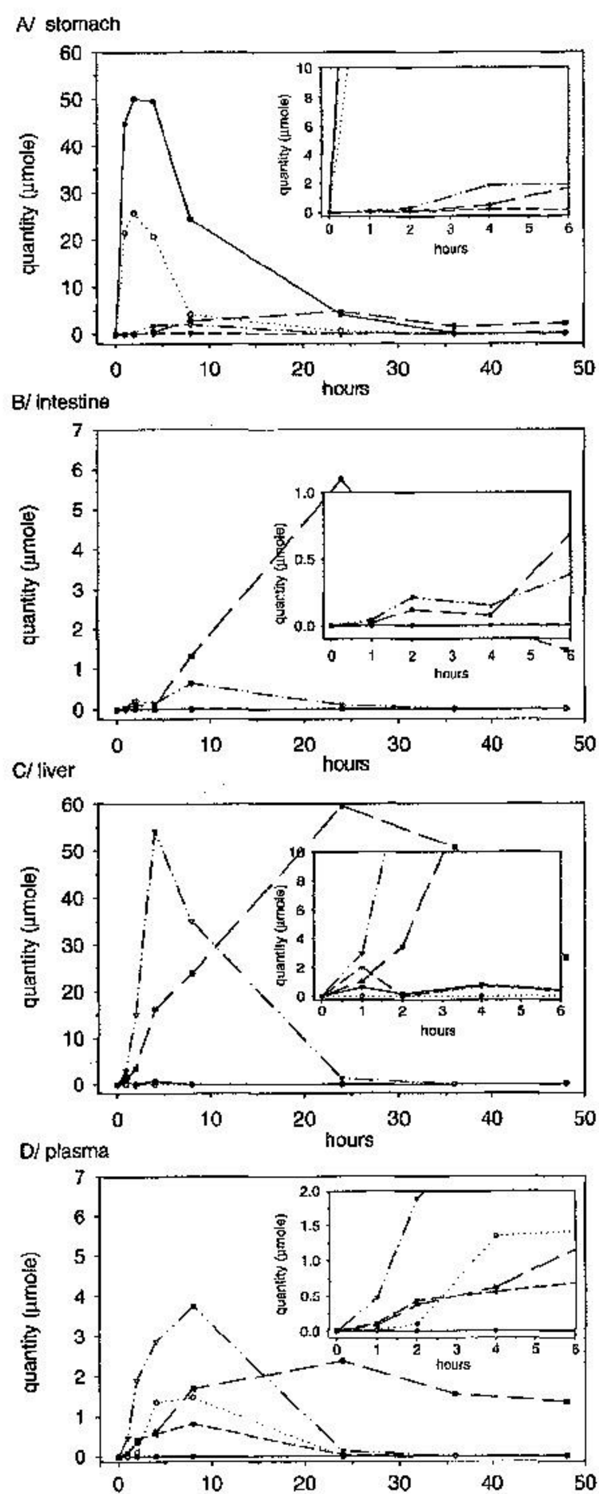


Figure 3. Quantity of volatile metabolites during the 48-h period in the stomach, intestine, liver, and plasma after one oral administration of $200\,mg\,kg^{-1}$ of DPDS to male rats. —●— DPDS, ·····o····· PM, — —▼— — MPS, · · —▽ · · — MPSO, —— ■— — $MPSO_2$, Results are the mean of three rats.

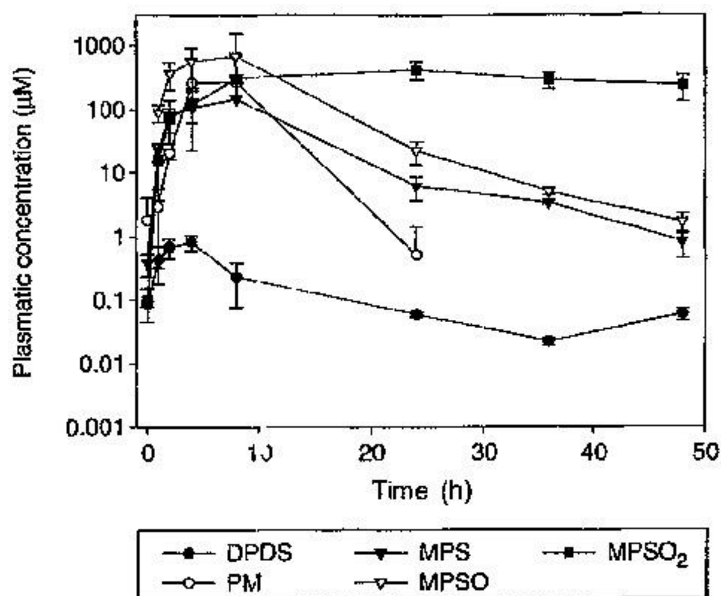


Figure 4. Plasma concentration–time curve of metabolites of DPDS after one oral administration to male rats. Data are means of three rats ± standard deviation.

Applicant's summary and conclusion

Interpretation of results : no bioaccumulation potential based on study results

Executive summary

The metabolism of dipropyl disulphide (DPDS), was investigated *in vivo* in the rat. A single dose (200 mg/kg)) was administered by gastric intubation and the time courses of DPDS and its metabolites were followed over 48 h by gas chromatography coupled with mass spectrometry in the stomach, intestine, liver, and blood.
DPDS was detected in the stomach where it was transformed into propyl mercaptan, whereas the liver contained only traces of DPDS and none at all in the other examined organs. The metabolites methylpropyl sulphide, methylpropyl sulphoxide (MPSO), and methylpropyl sulphone (MPSO2) were sequentially formed in the liver. The route of elimination from the liver seemed to be mainly via the blood. The bile also participated in the excretory process, but only for MPSO2.
The pharmacokinetic parameters were determined for all of the above compounds. Whereas the bioavailability of DPDS was very low (0.008 h mM), the areas under the curve were higher for the S-oxidized metabolites MPSO and MPSO2, i.e. 9.64 and 24.15 h mM, respectively. The half-lives for DPDS and its metabolites varied between 2.0 and 8.25 h, except for MPSO2, which had a half-life of 29.6 h. MPSO2 was the most abundant and persistent of these metabolites.

[Study 3] Kilford (Covance) 2012/K1 KS/in vitro metabolism

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable with restrictions)	Kilford P	2012	Dimethyl disulphide (DMDS): In vitro metabolism in hepatocytes from rats

Materials and methods

Type of method : in vitro

Objective of study: metabolism

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

To identify the number and proportions of its metabolites, dimethyl disulphide was incubated with fresh rat hepatocytes. Samples were removed after 0, 1, 2 and 4 hours and quenched in liquid nitrogen. All samples were analysed by GC-MS.

GLP compliance

no (Although a claim of GLP compliance has not been made for this study, the laboratory procedures were conducted in accordance with the current GLP requirements of the UK MHRA and OECD.)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.8%

Radiolabelling : no

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : male/female

Administration / exposure

Route of administration : other: in vitro

Vehicle : other: incubation medium

Any other information on materials and methods incl. tables

Materials and experimental Procedures
Dissolution of Test Substance
Stock solutions of Dimethyl disulphidewere prepared in incubation medium. The actual concentration of the formulation was determined after the incubation.

Reagents
General purpose reagents and solvents were of Analar grade (or a suitable alternative) and were obtained principally from VWR International Ltd, Rathburn Chemicals Ltd, Aldrich Chemical Company Ltd and Vickers Laboratories Ltd. Suitable liquid scintillants were obtained from Perkin Elmer Life Sciences Ltd.

Equipment
Radio-HPLC was performed on an Agilent 1100 chromatographic system with a Packard FSA 525TR radio-detector. HPLC data were captured on line with Laura software (version 3.4.7.52 SP8).

Test System
Source
Liver samples were obtained from one male and one female Sprague-Dawley rat. Hepatocytes were prepared by a two-step collagenase perfusion method.
The source of all tissue was recorded in the raw data and documented in the final report. The test system was identified by means of a label containing information such as species, lot number etc.

Cell Viability and [^{14}C]7-Ethoxycoumamarin Metabolism

Cell viability of the hepatocyte suspensions was estimated by trypan blue exclusion. Metabolic capacity of the suspension cultures was determined by quantification of [^{14}C]7-ethoxycoumarin metabolism.

Experimental Procedures
Cell Culture Medium
Leibovitz L-15 medium, pre-warmed to*ca.*37°C, was used for all incubations.

Control matrix analysis
A sample of the control rat hepatocyte matrix (1 x 10^6cells/mL) was supplied to the Department of Environmental Sciences for analysis along with the incubation samples.

Dimethyl disulphide Incubations
All incubations and GC analysis was carried out in Headspace vials.
Dimethyl disulphide was incubated at a nominal concentration of 10 µmol/L with freshly prepared rat hepatocytes in suspension in Leibovitz L-15 medium (1 x 10^6cells/mL). Incubations were performed at*ca.*37°C in a shaking water bath and terminated at 0, 1, 2 and 4 hours. The incubations were terminated by samples being frozen in liquid nitrogen. A blank incubation was carried out with either Leibovitz L-15 medium in the place of hepatocytes or in the place of DMDS.
Eight replicates at each time point were generated and supplied to the Department of Environmental Sciences and analysed by GC-MS.

[^{14}C]7-Ethoxycoumarin Incubations
[^{14}C]7-Ethoxycoumarin, at a nominal concentration of 50 µmol/L, was incubated in a suspension of freshly prepared rat hepatocytes at a nominal cell density of 1 x 10^6viable cells/mL in cell culture medium. Incubations were performed at*ca*37°C in a shaking water bath and terminated after 0 and 4 hours. All incubations were carried out in duplicate.
The formation of 7-hydroxycoumarin and the corresponding glucuronide and sulphate conjugates was quantified by radio-HPLC.

Sample analysis
Determination of 7-Ethoxycoumarin Metabolism
The formation of 7-hydroxycoumarin and the corresponding glucuronide and sulphate conjugates in the hepatocyte incubations was estimated by radio-HPLC.

Chemical Analysis
The concentration of Dimethyl disulphide and potential metabolites (dimethyl sulphide, dimethyl sulfone, dimethyl sulfoxide and methyl mercaptan) in the hepatocyte samples was measured using GC-MS.

Results and discussions

Metabolite characterisation studies

Metabolites identified : yes

Details on metabolites
Methyl mercaptan and dimethyl sulphide

Bioaccessibility

Any other information on results incl. tables
Hepatocyte Viabilityand Metabolic Capacity
The cell viabilities of the fresh hepatocytes as measured by trypan blue exclusion were 71% and 67% in male and female rat, respectively. The cells were considered acceptable for use and were pooled prior to use.
Phase I metabolic capacity was assessed by the quantitative appearance of 7-hydroxycoumarin following incubation of [^{14}C]7-ethoxycoumarin in suspensions of hepatocytes from rats (male and female pooled). Phase II activity was assessed by the quantitative appearance of the corresponding glucuronide and sulphate conjugates. The metabolic capacity assessment is shown inTable1. All hepatocyte preparations were found to have acceptable Phase I and Phase II metabolic capacity when compared to historical data.

Concentration of Dimethyl Disulphide in Hepatocyte incubations
The concentration of dimethyl disulphide was 588 and 177 nmol/L in the incubations with rat hepatocytes from replicate 3 and 4, respectively. This was lower than the anticipated concentration due to the volatile nature of handling the test compound. However, the concentration achieved still allowed the evaluation of the formation of the metabolites of dimethyl disulphide.

Incubation of Rat Hepatocytes in the Absence of Dimethyl Disulphide
Control incubations containing buffer instead of dimethyl disulphide were performed (Table2).
Following incubation of rat hepatocytes in the absence of dimethyl disulphide three peaks were observed at retention times corresponding to dimethyl disulphide, dimethyl sulphide and methyl mercaptan. Methyl mercaptan was the most abundant substance, whereas the 2 other metabolites were present at low levels. Therefore the concentrations in the control incubations were subtracted from the corresponding concentrations of parent and metabolites in the incubations with dimethyl disulphide and hepatocytes.

Incubation of Dimethyl Disulphide in the Absence of Hepatocytes
Control incubations containing buffer instead of hepatocytes were performed (Table3).
Following incubation of dimethyl disulphide in the absence of hepatocytes for 4 hours only dimethyl disulphide and methyl mercaptan were detected in the incubate samples.
As dimethyl disulphide is hydrolytically stable it is likely that methyl mercaptan was formed during the analysis, however, this could not be confirmed from data.

Incubation of Dimethyl Disulphide with Rat Hepatocytes
Dimethyl disulphide was metabolised extensively in fresh rat hepatocytes with the parent molecule concentrations of 66.0 and 92.3 nmol/L after 4 hours for replicate 3 and 4, respectively (Table4). As the amount of dimethyl disulphide decreased, the levels of metabolites were observed to increase at all time points
Two metabolites, dimethyl sulphide (DMS) and methyl mercaptan, were observed at measurable levels, greater than background levels in fresh rat hepatocytes after incubation for 4 hours. The major metabolite was attributed to methyl mercaptan with concentrations of 472 and 738 nmol/L at 4 hours in replicate 3 and 4, respectively (Table4).
Notably, the formation of the oxidation products of DMS, dimethylsulfoxide (DMSO) and dimethylsulfone ($DMSO_{2}$)was not observed during the incubation with fresh rat hepatocytes.

Overall remarks, attachments

Remarks on results including tables and figures
Table 1
Metabolic Capacity Assessment of Hepatocytes (Quantitative Assessment of the Appearance of 7-Ethoxycoumarin Metabolites Over 4 Hour Incubation)

Sample ID	Total recovery of radioactivity (%)	% Chromatogram radioactivity				
		7-Ethoxy-coumarin	Glucuronide*	7-Hydroxy-coumarin	Sulphate*	Other metabolites**
Rat: 0hr Rep 1	95.5	93.5	ND	ND	ND	6.49
Rat: 0hr Rep 2	90.2	99.0	ND	ND	ND	0.37
Mean	**92.9**	**96.2**	**NA**	**NA**	**NA**	**3.43**
Rat: 4hr Rep 1	90.6	8.19	11.5	10.9	19.0	49.5
Rat: 4hr Rep 2	89.1	6.87	2.16	2.97	24.8	63.2
Mean	**89.8**	**7.53**	**6.82**	**6.94**	**21.9**	**56.4**

NA - not applicable
ND – not detected
* of 7-hydroxycoumarin
** Sum of other metabolite peaks present in chromatogram

Table 2
Peaks observed following incubation with fresh rat hepatocytes in the absence of Dimethyl disulphide for up to 4 hours

Sample	Replicate	Time (hours)	Concentration (nmol/L)		
			DMDS*	DMS*	MeS*
Control	-	-	31.9	24.0	1746
TA Blank	3	0	31.5	35.5	573
		4	49.2	36.9	623
		Mean	**40.3**	**36.2**	**598**
TA Blank	4	0	110	37.3	1095
		4	71.0	36.9	831
		Mean	**90.4**	**37.1**	**963**

TA – Test article
DMDS – Dimethyl disulphide
DMS – Dimethyl sulphide
MeS – Methyl mercaptan
*Observed peaks due to matrix interferences with retention times corresponding to the peaks of interest

Table 3
Metabolites observed following incubation of Dimethyl disulphide in the absence of hepatocytes for up to 4 hours

Sample	Replicate	Time (hours)	Concentration (nmol/L)		
			DMDS	DMS	MeS
Matrix Blank	3	0	199	ND	8.77
		4	137	ND	26.4
		Mean	**166**	**NA**	**17.6**
Matrix Blank	4	0	1375	ND	211
		4	687	ND	150
		Mean	**1031**	**NA**	**180**

ND – Not detected
NA – Not applicable
DMDS – Dimethyl disulphide
DMS – Dimethyl sulphide
MeS – Methyl mercaptan

Table4
Metabolites observed following incubation of Dimethyl disulphide with fresh rat hepatocytes for up to 4 hours

Sample	Replicate	Time (hours)	Concentration (nmol/L)*		
			DMDS	DMS	MeS
Rat	3	0	588	4.44	364
		1	63.7	8.15	500
		2	ND	ND	ND
		4	66.0	5.24	442
Rat	4	0	171	0.16	ND
		1	48.6	0.16	ND
		2	83.1	13.1	825
		4	92.3	12.1	691

ND – Not detected
DMDS – Dimethyl disulphide
DMS – Dimethyl sulphide
MeS – Methyl mercaptan
*Corrected for interferences in test article blanks

Illustration (picture/graph)

Covance Study Number: 8260134
Final Report

Figure 1 **Metabolism of Dimethyl Disulphide After Incubation With Fresh Rat Hepatocytes for up to 4 hours – Replicate 3 Samples**

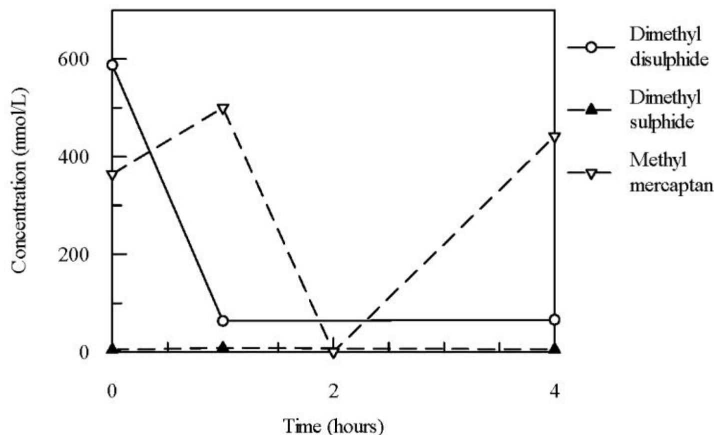
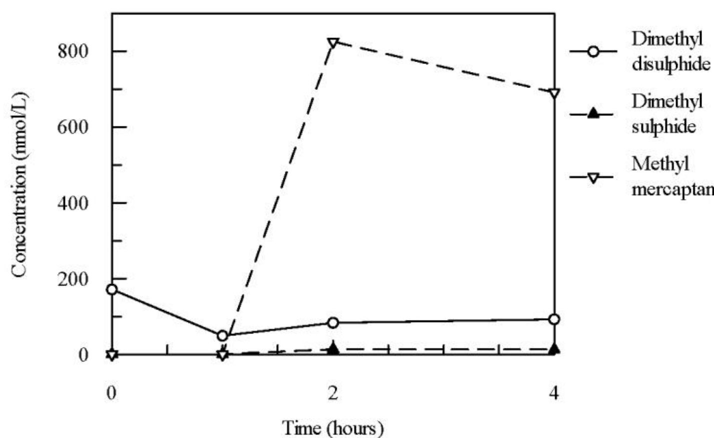


Figure 2 **Metabolism of Dimethyl Disulphide After Incubation With Fresh Rat Hepatocytes for up to 4 hours – Replicate 4 Samples**



- 17 -

Applicant's summary and conclusion

Interpretation of results : bioaccumulation potential cannot be judged based on study results

Conclusions

Dimethyl disulphide, dimethyl sulphide and methyl mercaptan are present in the control hepatocyte solutions
Dimethyl disulphide was metabolised rapidly after incubation with fresh rat hepatocytes for up to 4 hours

Formation of both dimethyl sulphide and methyl mercaptan was observed over the 4 hour incubation
No formation of DMSO or DMSO2 was observed during the incubation with fresh rat hepatocytes.

Executive summary

The objective of this study was to identify the number and proportions of the metabolites generated following incubation of dimethyl disulphide with fresh rat hepatocytes using a GC-MS method. Dimethyl disulphide was incubated with fresh rat hepatocytes. Samples were removed after 0, 1, 2 and 4 hours and quenched in liquid nitrogen. All samples were analysed by GC-MS. [^{14}C]-7-ethoxycoumarin was also incubated with rat hepatocytes suspended in Leibovitz L-15 medium. Samples were terminated after 0 and 4 hours and all samples quantified by radio-HPLC for the formation of 7-hydroxycoumarin and glucuronide and sulphate conjugates. Dimethyl disulphide was metabolised rapidly after incubation with fresh rat hepatocytes for up to 4 hours. Methyl mercaptan was the main metabolite observed over the 4 hour incubation, dimethyl sulphide (DMS) was found at a low level. No formation of the oxidation products of DMS, dimethylsulfoxide (DMSO) or dimethylsulfone ($DMSO_2$) was observed during the incubation with fresh rat hepatocytes.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

[Study 1] Pelcot (CIT) 2010/ K1 KS / Acute oral toxicity: rat

Study reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
publication	Key study	1 (reliable with restrictions)	Pelcot C	2010	Dimethyl disulphide. Acute oral toxicity in rats, "acute toxic class method"

Materials and methods

Test type : acute toxic class method

Limit test : yes

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method)	no
according to	EU Method B.1 tris (Acute Oral Toxicity - Acute Toxic Class Method)	no

GLP compliance : yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.88%

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Janvier, Le Genest-Saint-Isle, Franc
- Age at study initiation: approximately 8 and 9 weeks old
- Weight at study initiation: 212 ± 15 g.
- Fasting period before study: overnight period of approximately 18 hours before dosing
- Housing: 3 in polycarbonate cages with stainless steel l
- Diet (e.g. ad libitum): SSNIFF R/M-H pelleted maintenance diet (SSNIFF Spezialdiäten GmbH, Soest, Germany)
- Water (e.g. ad libitum): filtered by a FG Millipore membrane (0.22 micron)
- Acclimation period: at least 5 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22 ± 2
- Humidity (%): 30 to 70
- Air changes (per hr): 12
- Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration : oral: gavage

Vehicle : corn oil

Details on oral exposure

VEHICLE
- Concentration in vehicle: 30 mg/ml
- Justification for choice of vehicle: solubility
- Lot/batch no. (if required): 098K0008
- Purity: no data

MAXIMUM DOSE VOLUME APPLIED: 10 ml/kg

CLASS METHOD (if applicable)
- Rationale for the selection of the starting dose: 300 mg/kg is the limit dose for classification as toxic according to CLP classification

Doses : 300 mg/kg

No. of animals per sex per dose : 6

Control animals : no

Details on study design

CLINICAL EXAMINATIONS

The single administration was performed in the morning of day 1; it was followed by a 14-day observation period.

Clinical signs and mortality:The animals were observed frequently during the hours following administration of the test item, for detection of possible treatment-related clinical signs. Thereafter, observation of the animals was made at least once a day.Type, time of onset and duration of clinical signs were recorded for each animal individually.

Body weight:The animals were weighed individually just before administration of the test item on day 1 and then on days 8 and 15.The body weight gain of the treated animals was compared to that of CIT control animals with the same initial body weight.

PATHOLOGY

Sacrifice:On day 15, all animals will be deeply anesthetized by an intraperitoneal injection of sodium pentobarbital and sacrificed by exsanguination.

Macroscopic necropsy examination:All study animals were subjected to a macroscopic examination as soon as possible after death. After opening the thoracic and abdominal cavities, a macroscopic examination of the main organs (digestive tract, heart, kidneys, liver, lungs, pancreas, spleen and any other organs with obvious abnormalities) was performed.

Statistics

Not appropriate for a limit test

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
female	LD0	>= 300 mg/kg bw			no mortality, clinical signs included hypoactivity, hypersalivation, piloerection and dyspnea

Mortality : No deaths occurred.

Clinical signs

Hypoactivity, dyspnea and hypersalivation were noted in all animals 10 minutes after treatment. Hypoactivity persisted in 4/6 animals until 4 hours after treatment. In addition, piloerection and dyspnea were observed in one animal 3 hours after treatment and in another one 4 hours after treatment.

Body weight

When compared to historical control animals, a slightly lower body weight gain was noted in 3/6 animals between day 1 and day 8, persisting in one of them between day 8 and day 15. The body weight gain of the other animals was not affected by treatment with the test item.

Gross pathology

Macroscopic examination at the end of the observation period of the main organs of the animals revealed no apparent abnormalities.

Applicant's summary and conclusion

Interpretation of results : Toxicity Category IV

Criteria used for interpretation of results

other: REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008

Conclusions

The oral LD0 of DIMETHYL DISULPHIDE was higher than 300 mg/kg in rats.

Executive summary

The acute oral toxicity of neat dimethyl disulphide was evaluated in rats according to OECD No. 423 (17th December 2001) and Council Regulation (EC) No. 440/2008, B.1*tris*(30 May 2008) guidelines. The study was conducted in compliance with the principles of Good Laboratory Practice Regulations. Dimethyl disulphide was administered in corn oil by oral route (gavage), under a volume of 10 mL/kg to 6 fasted female Sprague-Dawley rats. Clinical signs, mortality and body weight gain were checked for a period of up to 14 days following the single administration. All animals were subjected to necropsy.
No deaths occurred. Hypoactivity, dyspnea and hypersalivation were noted in all animals 10 minutes after treatment. Hypoactivity persisted in 4/6 animals until 4 hours after treatment. In addition, piloerection and dyspnea were observed in one animal 3 hours after treatment and in another one 4 hours after treatment.
When compared to historical control animals, a lower body weight gain was noted in 3/6 animals between day 1 and day 8, persisting in one of them between day 8 and day 15. The body weight gain of the other animals was not affected by treatment with the test item.The oral $LD_0$of dimethyl disulphide was higher than 300 mg/kg in rats.

[Study 2] Lombard (CIT) 1986/K1 SS/Acute oral toxicity, rat

Study reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	supporting study	1 (reliable with restrictions)	Lombard A	1986	Dimethyl disulfure (DMDS) - Evaluation de la toxicité aigüe chez le rat par voie orale

Materials and methods

Test type : standard acute method

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
according to	EU Method B.1 (Acute Toxicity (Oral))	no

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.31%

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River France
- Age at study initiation: no data
- Weight at study initiation: 141-148 g (males) , 120-126 g (females)
- Fasting period : 18 hours before and 4 hours after treatment
- Housing: 5 per polycarbonate cage
- Diet : rat and mouse maintenance diet A 04C (UAR, Villemeoisson/orge, France), ad libitum
- Water : filtrated tap water, ad libitum
- Acclimation period: at least 7 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22 +/- 3°C
- Humidity (%): 50 +/- 20%
- Ventilation : filtered, not recycled air
- Photoperiod (hrs dark / hrs light): 12/12 h

IN-LIFE DATES: from 27/02/1986 to 02/04/1986

Administration / exposure

Route of administration : oral: gavage

Vehicle : other: polyethylene glycol 300

Details on oral exposure

DIMETHYL DISULFIDE was administered undiluted at a volume of 5 ml/kg bw, or as a suspension (10 ml/kg) in

polyethylene glycol 300 at the dose levels of 100, 170, 290, 350 and 500 mg/kg.

Doses : 100, 170, 290, 350, 500 mg/kg and 5 ml/kg (5300 mg/kg)

No. of animals per sex per dose : 5

Control animals : no

Details on study design

- Duration of observation period following administration: 14 days
- Frequency of clinical observations: frequently following dosing then daily- Mortality: twice daily
- Frequency of weighing: on days 0, 5, 8 and 15
- Necropsy of survivors performed: yes

Results and discussions

Effect levels

Sex	**Endpoint**	**Effect level**	**Based on**	**95% CL**	**Remarks**
male/female	LD50	> 290 — < 500 mg/kg bw			Post-exposure clinical signs: sedation, hypotonia, dyspnea, piloerection and coma
male/female	approx. LD50	385 mg/kg bw		336 — 752	LD50 estimated with a standard probit method

Mortality

- 100 and 170 mg/kg : none
- 290 mg/kg : 30 %
- 350 mg/kg : none
- 500 mg/kg : 100 %
- 5 ml/kg : 100 % after 10 minutes, due to a significant regurgitation

Clinical signs

100 and 170 mg/kg: sedation, dyspnea and piloerection between 5 min and 4h post treatment. No mortality. No clinical signs from D2 to D15.290 mg/kg: sedation, dyspnea, hypotonia, piloerection, and mortality between 5 min and 4h post treatment. No clinical signs from D2 to D15 in surviving animals.350 mg/kg: sedation between 5 min and 3h post treatment. No mortazlity. No clinical signs from D2 to D15.500 mg/kg: sedation, hypotonia, dyspnea, and mortality between 5 and 30 min post exposure.

Body weight

No effect was noted on the body weight gain of the surviving rats.

Gross pathology

Haemorragic stomachs with foam in the stomach and the trachea was observed at the macroscopic examination of the rats dead on the first day at 290 (one male and 2 females) and 500 mg/kg (3 males and 3 females). Haemorragic stomachs at 500 mg/kg in 2 males and 2 females. No abnormalities were observed in the animal sacrificed at the end of the observation period.

Applicant's summary and conclusion

Interpretation of results : harmful (Category 4)

Criteria used for interpretation of results

other: REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008

Conclusions

Under the experimental conditions of this study, the oral LD50 of DMDS was between 290 and 500 mg/kg in rats and an approximate LD50 of 385 mg/kg was calculated according to the standard probit method by the Notifier.

Executive summary

The Acute oral toxicity of dimethyl disulphide (DMDS) was evaluated in male and female Sprague-Dawley rats according to EU Method B.1.Animals were treated with dose levels of 100, 170, 290, 350, 500 and 5300 mg/kg (5 ml/kg) and then observed for 14 days for mortality, clinical signs and effect on body weight.
No mortality was observed at the dose levels of 100, 170 and 350 mg/kg. At 290 mg/kg the mortality was 30% and 100% at 500 and 5300 mg/kg. Sedation, hypotonia, dyspnea, piloerection and coma, appeared just after the administration and disappeared after 24 hours. No effect was noted on the body weight gain of the surviving rats. Haemorragic stomachs was observed at the macroscopic examination of the rats dead on the first day (290 and 500 mg/kg)
Under these experimental conditions, the oral LD50 of DMDS is between 290 and 500 mg/kg in female and male rats and an approximate LD50 of 385 mg/kg was calculated according to the standard probit method.

[Study 3] Gilotti (MB Research) 2006 / K1 / Acute oral toxicity: rat

Study reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report		1 (reliable with restrictions)	Gilotti AC	2006	TD-2474-02: Acute Toxic Class Determination (Oral)

Materials and methods

Test type : acute toxic class method

Limit test : yes

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method)	yes (the observation period was terminated 6 days after the treatment)

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 93.3%

Test animals

Species : rat

Strain : Wistar

Sex : female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Ace Animals, Boyertown, PA, USA
- Age at study initiation: 9-week old
- Weight at study initiation: 153 - 163 grams
- Fasting period before study: no data
- Housing: in suspended wire mesh cages; 5/sex/cage prior to dosing and 3/sexlcage following dosing
- Diet (e.g. ad libitum): PMI Rat Chow (Diet #5012)
- Water (e.g. ad libitum): tap water

- Acclimation period: at least five days
ENVIRONMENTAL CONDITIONS
- Temperature (°C): controlled
- Humidity (%): no data
- Air changes (per hr): no data
- Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration : oral: gavage

Vehicle : unchanged (no vehicle)

Details on oral exposure

The test article was used as received and the dose was based on the sample weight as calculated from the specific gravity

Doses : 501 mg/kg

No. of animals per sex per dose : 3

Control animals : no

Details on study design

The survivor was observed 1/2, 1, 2, 3 and 4 hours postdose and once daily for 6 days for mortality, toxicity and pharmacological effects. Body weights for ail animals were recorded immediately pretest.The survivor was humanely sacrificed using C02 and was not examined for gross pathology following study termination. The two animais that died were examined for gross pathology. Abnormal tissues were preserved in 10% neutral buffered formalin for possible future histological examination.

Statistics

Not appropriate for a limit test

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
female	LD50	< 501 mg/kg bw			

Mortality

One of three animals survived the 501 mg/kg oral dose to Day 6 at which time the animal was sacrificed at the request of the sponsor. Two animals died prior to the 1/2 hour observation period

Clinical signs

The sacrificed animal was noted with chromorhinorrhea at 1/2 hour postdose and bloated abdomen and alopecia on forelimbs on Day 6. All other observations in the survivor were normal.

Body weight

No data, the animals died or were sacrificed before day 7.

Gross pathology

Necropsy results of theee animals revealed abnormalities of the Iungs, liver, stomach and intestines.

Applicant's summary and conclusion

Conclusions

The oral LD50 of dimethyl disulphide was lower than 501 mg/kg in rats.

Executive summary

The acute oral toxicity of dimethyl disulphide was evaluated in rats according to OECD No. 423 (17th December 2001) guideline. The study was conducted in compliance with the principles of Good Laboratory Practice Regulations. Dimethyl disulphide EC (94% DMDS with emulsifier and odorant) was administered undiluted by oral route (gavage)to 3 female Wistar rats. Clinical signs, mortality and body weight gain were checked for a period of up to 6 days following the single administration. All animals were subjected to necropsy.
One of three animals survived the 501 mg/kg oral dose to Day 6 at which time the animal was sacrificed. Two animals died prior to the 0.5 hour observation period. Necropsy results of these animals revealed abnormalities of the lungs, liver, stomach and intestines. The sacrificed animal was noted with chromorhinorrhea at 0.5 hour postdose and bloated abdomen and alopecia on forelimbs on Day 6. All other observations in the survivor were normal.The oral $LD_{50}$of dimethyl disulphide was lower than 501 mg/kg in rats.

[Study 4] Gilotti (MB Research) 2007 / K1 / Acute oral toxicity: rat

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report		1 (reliable with restrictions)	Gilotti AC	2007	Dimethyl Disulfide 94:5:1. Acute Toxic Class Determination (Oral)

Materials and methods

Test type : acute toxic class method

Limit test : yes

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method)	yes (the observation period was terminated 6 days after the treatment)

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 94%

Test animals

Species : rat

Strain : Wistar

Sex : male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Ace Animals, Boyertown, PA, USA
- Age at study initiation: 8-week old
- Weight at study initiation: 172-227 grams
- Fasting period before study: no data
- Housing: in suspended wire mesh cages; 5/cage prior to dosing and 1/cage following dosing
- Diet (e.g. ad libitum): PMI Rat Chow (Diet #5012)
- Water (e.g. ad libitum): tap water
- Acclimation period: at least five days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): controlled
- Humidity (%): no data
- Air changes (per hr): no data
- Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration : oral: gavage

Vehicle : unchanged (no vehicle)

Details on oral exposure

The test article was used as received and the dose was based on the sample weight as calculated from the specific gravity. The test article was administered orally by syringe and dosing needle at a dose level of 500 mg/kg to five female rats, and at a dose level of 56 mg/kg to five female and five male rats.

Doses : 500 and 56 mg/kg bw

No. of animals per sex per dose : 5

Control animals : no

Details on study design

The survivor was observed 0.5, 1, 2 and 4 hours postdose and once daily for 14 days for mortality, toxicity and pharmacological effects. Body weights for ail animals were recorded immediately pretest, weekly and at termination.The survivor was humanely sacrificed using C02 and was not examined for gross pathology following study termination. Abnormal tissues were preserved in 10% neutral buffered formalin for possible future histological examination.

Statistics

Not appropriate for a limit test

Results and discussions

Effect levels

Sex	**Endpoint**	**Effect level**	**Based on**	**95% CL**	**Remarks**
female	LD100	500 mg/kg bw			No animals survived. Predeath signs: wetness of the nose/mouth area, flaccid muscle tone, prostration, negative righting reflex and coma. At necropsy: abnormalities of the lungs, thymus and liver, as well as wetness of the nose/mouth area.
male/female	LD0	> 56 mg/kg bw			No mortality. Instances of wetness of the nose/mouth area, lethargy, sagging eyelids, negative righting reflex, ataxia and wetness of the anogenital area in the males not in the females

Mortality

No animals survived the 500 mg/kg oral dose, death occurred within 70 minutes of dosing. Ten of ten animals survived the 56 mg/kg oral dose.

Clinical signs

500 mg/kg: Predeath signs included wetness of the nose/mouth area, flaccid muscle tone, prostration, negative righting reflex and coma.56 mg/kg: Instances of wetness of the nose/mouth area, lethargy, sagging eyelids, negative righting reflex, ataxia and wetness of the anogenital area were noted in the males within 2 hours postdose. Otherwise, the males appeared normal for the duration of the study. Females appeared normal throughout the study.

Body weight

56 mg/kg: Body weight changes were normal

Gross pathology

500 mg/kg: Necropsy results revealed abnormalities of the lungs, thymus and Iiver, as well as wetness of the nose/mouth area.
56 mg/kg: All animais appeared normal at necropsy.

Applicant's summary and conclusion

Conclusions

The LD50 of Dimethyl Disulfide 94:5:1 is greater than 56 mg/kg but less than 500 mg/kg.

Executive summary

The acute oral toxicity of dimethyl disulphide was evaluated in rats according to OECD No. 423 (17th December 2001) guideline. The study was conducted in compliance with the principles of Good Laboratory Practice Regulations. Five healthy female Wistar albino rats were dosed orally with Dimethyl Disulfide 94:5:1 (94% DMDS with emulsifier and odorant) at 500 mg/kg. All five rats dosed at 500 mg/kg died by the 2 hour observation period. Five females and five males were dosed at 56 mg/kg. Animals were observed 0.5, 1, 2 and 4 hours postdose and once daily for 14 days for mortality, toxicity and pharmacological effects. Body weights were recorded immediately pretest, weekly and at termination. All animals were examined for gross pathology. Abnormal tissues were preserved in 10% neutral buffered formalin for possible future histological examination. At 500 mq/kg, no animals survived. Death occurred within 70 minutes of dosing. Predeath signs included wetness of the nose/mouth area, flaccid muscle tone, prostration, negative righting reflex and coma. Necropsy results revealed abnormalities of the lungs, thymus and liver, as well as wetness of the nose/mouth area. At 56 mg/kg, ten of ten animais survived. Instances of wetness of the nose/mouth area, lethargy, sagging eyelids, negative righting reflex, ataxia and wetness of the anogenital area were noted in the males within 2 hours postdose. Otherwise, the males appeared normal for the duration of the study. Females appeared normal throughout the study. Body weight changes were normal. All animals appeared normal at necropsy. The LD50 of Dimethyl Disulfide 94:5:1 is greater than 56 mg/kg but less than 500 mg/kg.

[Study 5] Shapiro (PSL) 1985/K4/Acute oral toxicity, rat

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Disregarded study	4 (not assignable)	Shapiro R	1985	DIMETHYL DISULFIDE. EPA acute LD50

Materials and methods

Test type : standard acute method

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: EPA 40 CFR 163.81-1	no

GLP compliance : yes

Test materials

DMDS tested in this study did not correspond to the specifications of DMDS proposed for registration (high level of methyl mercaptan).

Test animals

Species : rat

Strain : Wistar

Sex : male/female

Details on test animals and environmental conditions

TEST ORGANISMS:
- Source: Royalhart Colony, Newhampton, NY
- Age: no data
- Weight at study initiation: 163-269 g
- Adaptation period: 14 days
- Fasting before treatment: 18 hours
- Adaptation period : 14 days

HOUSING : The animals were housed individually in suspended stailess steel wire-bottomed cages

FOOD and WATER
- Food: Purina rat chow ad libitum
- Water: ad libitum

ENVIRONMENTAL CONDITIONS
- Temperature : 68-72°F
- Relative humidity : no data
- Light/dark cycle : 12h/12h
- Ventilation : no data

Administration / exposure

Route of administration : oral: gavage

Vehicle : other: 3% CMC (carboxymethyl cellulose)

Details on oral exposure

- Volume administered: no data

Doses : 125, 188, 250, 375 and 500 mg/kg

No. of animals per sex per dose : 5

Control animals : no

Details on study design

Clinical signs, mortality and body weight gain were checked for a period of up to 14 days following the single administration of the test item. All animals were subjected to necropsy.

Statistics

Litchfield-Wilcoxon method of probit analysis.

Results and discussions

Effect levels

Sex	**Endpoint**	**Effect level**	**Based on**	**95% CL**	**Remarks**
male/female	LD50	190 mg/kg bw		150 — 240	Post-exposure clinical signs: sedation, hypotonia, dyspnea, piloerection and coma

Mortality

Group	Dose g/kg	Mortality Male	Mortality Female	Mortality %
1	0.125	0/5	1/5	10
2	0.188	5/5	1/5	60
3	0.250	3/5	4/5	70
4	0.375	5/5	5/5	100
5	0.50	5/5	5/5	100

Clinical signs

Dose Level 0.125 g/kg: One female died approximately 2-1/2 hours after administration of the test material. The second female did not exhibit any movement on the day of dosing, but appeared to be recovered by the next morning, and did not exhibit any signs of toxicity for the duration of the test. All other rats did not exhibit any signs of toxicity during the test period.
Dose Level 0.188 g/kg: Approximately 2-1/2 hours after administration of the test material, 5 male rats and 1 female rat died. The remaining 4 female rats exhibited signs of excessive salivation and lethargy. The rats exhibited signs of lethargy through day 5 post-dosing. From day 6 through 14 the rats did not exhibit any signs of toxicity.
Dose Level 0.250 g/kg: Approximately 2-1/2 hours after administration of the test material, 3 male and 4 female rats died. The surviving rats exhibited signs of lethargy and watery ocular discharge. The rats maintained hunched positions. By the morning of day 1, the rats had recovered and did not exhibit any signs of toxicity throughout the duration of the test.
Dose Level 0.375 g/kg: All animals died approximately 2-1/2 hours after administration of the test material.
Dose Level 0.50 g/kg: All animals died approximately 2-1/2 hours after administration of the test material

Body weight

Dose Level 0.125, 1.188 and 0.250 g/kg: All surviving rats gained weight at 7 and 14 days post-dosing.

Gross pathology

Dose Level : 0.125 g/kg - Group 1: The necropsy of the 1 mortality showed evidence of pulmonary hemorrhage and an enlarged spleen. Necropsy of the survivons was unrevealing. All organs and tissues appeared normal.
Dose Level 0.188 g/kg: Necropsy of the mortalities on the day of dosing revealed evidence of pulmonary hemorrhage and one animal exhibited an enlarged spleen. Necropsy of the survivons was unreveallng. All organs and tissues appeared normal.
Dose Level 0.250 g/kg: Necropsy of the mortalities revealed evidence of pulmonary hemorrhage. One male exhibited gastro-intestinal hemorrhage and an enlarged spleen. Necropsy of survivors was unreveallng. All organs and tissues appeared normal.
Dose Level 0.375 g/kg: The necropsy revealed evidence of pulmonary hemorrhage in all but 2 rats. One male exhibited an enlarged spleen, and 1 female exhibited a gastro-intestinal hemorrhage. One male and 1 female did not exhibit any abnormal organs or tissues.
Dose Level 0.50 g/kg: 5 All animals died approximately 2-1/2 hours after administration of the test material. The necropsy revealed evidence of pulmonary hemorrhage in all animalis. One male and 1 female exhibited enlarged spleens, and 2 females exhibited gastro-intestinal hemorrhages.

Applicant's summary and conclusion

Executive summary

The Acute oral toxicity of DIMETHYL DISULFIDE was evaluated in male and female Wistar rats according to EPA 40 CFR 163.81-1and in compliance with principles of Good Laboratory Practices. Animals were treated with dose levels of 125, 188, 250, 375 and 500 mg/kg and then observed for 14 days for mortality, clinical signs and effect on body weight.
According to the dose levels, mortality was 10, 60, 70, 100 and 100% respectively.
Sedation, hypotonia, dyspnea, piloerection and coma, appeared just after the administration and disappeared after 24 hours. No effect was noted on the body weight gain of the surviving rats. Haemorragic stomachs was observed at the macroscopic examination of the rats dead on the first day (250 and 500 mg/kg)
Under these experimental conditions, the oral LD50 of DMDS was 190 (150 -240) mg/kg in female and male rats.

[Study 6] Shapiro (PSL) 1985/K4/Acute oral toxicity, rat (limit test)

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Disregarded study	4 (not assignable)	Shapiro R	1985	DIMETHYL DISULFIDE, EPA Acute oral toxicity - limit test

Materials and methods

Test type : standard acute method

Limit test : yes

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: EPA 40 CFR 163.81-1	no

GLP compliance : yes

Test materials

DMDS tested in this study did not correspond to the specifications of DMDS proposed for registration (high level of methyl mercaptan).

Test animals

Species : rat

Strain : Wistar

Sex : male/female

Details on test animals and environmental conditions

TEST ORGANISMS:
- Source: Royalhart Colony, Newhampton, NY
- Age: no data
- Weight at study initiation: 216-230 g
- Adaptation period: 14 days

HOUSING The animals were housed individually in suspended stailess steel wire-bottomed cages

FOOD and WATER
- Food: Purina rat chow ad libitum
- Water: ad libitum

ENVIRONMENTAL CONDITIONS
- Temperature : 68-72°F
- Relative humidity : no data
- Light/dark cycle : 12h/12h
- Ventilation : no data

IN LIFE PHASE: April 10th, 1985

Administration / exposure

Route of administration : oral: gavage

Vehicle : other: none

Details on oral exposure

Following an adaptation period of 14 days, five male and five female rats were fasted for 18 hours and weighed. Rats were uniquely identified and dosed by gavage with the test substance (neat) at a rate of 5.0 g/kg body weight. A 3 inch 18 gauge intubation needle attached to a calibrated syringe, was used for dosing.

Doses : 5000 mg/kg

No. of animals per sex per dose : 5

Control animals : no

Details on study design

After dosing, the rats were observed for signs of gross toxicity and behavioral abnormalities during 14 days. All mortalities were weighed and autopsied as close to the time of death as possible.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
male/female	LD100	< 5000 mg/kg bw			Thirty minutes after the administration, all the animals were dead.

Mortality
100%

Clinical signs
Ten minutes after administration of the test material, animals began exhibiting signs of toxicity. The animals were gasping and laying on their sides. Thirty minutes later, all the animals were dead.

Body weight
-

Gross pathology
Necropsy of the mortalities revealed evidence of pulmonary hemorrhages, enlarged spleens in 1 male and 1 female, and a gastro-intestinal hemorrhage in 1 male.

Applicant's summary and conclusion

Conclusions
The acute oral toxicity of Dimethyl Disulfide is < 5.0 g/kg body weight.

3.2 Acute toxicity - dermal route

[Study 1] Gilotti (MB Research) 2007 / K1 WoE / Acute dermal toxicity: rat

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Weight of evidence	1 (reliable with restrictions)	Gilotti AC	2007	Dimethyl Disulfide 94:5:1, Acute Dermal Toxicity/LD50 in Rats

Materials and methods

Test type : standard acute method

Limit test : yes

Test guideline

Qualifier	Guideline	Deviations
according to	EPA OPPTS 870.1200 (Acute Dermal Toxicity)	no

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 94%

Test animals

Species : rat

Strain : Wistar

Sex :
male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Ace Animals, Boyertown, PA, USA
- Age at study initiation: 8-9-week old
- Weight at study initiation: 216 - 264 g for males and 202 - 231 g for females
- Fasting period before study: no data
- Housing: individually in suspended wire mesh cages
- Diet (e.g. ad libitum): PMI Rat Chow (Diet #5012)
- Water (e.g. ad libitum): tap water
- Acclimation period: at least one week

ENVIRONMENTAL CONDITIONS
- Temperature (°C): controlled
- Humidity (%): no data
- Air changes (per hr): no data
- Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Type of coverage : semiocclusive

Vehicle : unchanged (no vehicle)

Details on dermal exposure

TEST SITE
- Area of exposure: dorsal area of the trunk- % coverage: approximately 10% of the body surfac- Type of wrap if used: 4 ply porous gauze dressing measuring 10 x 15 cm wrapped with plastic in a semi-occlusive manner and was secured with non-irritating tape

REMOVAL OF TEST SUBSTANCE
- Washing (if done): washed with distilled water- Time after start of exposure: 24 hours

Duration of exposure : 24 hours

Doses : 5000 mg/kg

No. of animals per sex per dose :
5

Control animals : no

Details on study design

- Duration of observation period following administration: 14 days
- Frequency of observations and weighing:The test sites were scored for dermal irritation at 24 hours postdose and on days 7 and 14 using the numerical Draize scoring. The skin was also evaluated for ulceration and necrosis or any evidence of tissue destruction. Additional signs were described. The animals were observed 1, 2 and 4 hours postdose and once daily for 14 days for toxicity and pharmacological effects. The animals were observed twice daily for 14 days for mortality. Body weights were recorded pretest, weekly and at termination.
- Necropsy of survivors performed: yesAll animals were humanely sacrificed using CO_2 following study termination and were examined for gross pathology. Abnormal tissues were preserved in 10% neutral buffered formalin for possible future histological examination.

Statistics : Not appropriate for a limit test

Results and discussions

Effect levels

Sex	**Endpoint**	**Effect level**	**Based on**	**95% CL**	**Remarks**
male/female	LD0	> 5000 mg/kg bw			No mortality. Instances of wetness and soiling of the anogenital area, chromorhinorrhea, sagging eyelids, emaciated appearance, few feces, lethargy, ataxia, wet red substance on anogenital area and nose/mouth area were noted during the study

Mortality

All ten animals survived the 5000 mg/kg dermal application.

Clinical signs

Instances of wetness and soiling of the anogenital area, chromorhinorrhea, sagging eyelids, emaciated appearance, few feces, lethargy, ataxia, wet red substance on anogenital area and nose/mouth area were noted during the study.

Body weight

Two animals lost weight form Day 0 to Day 7, but gained weight overall by study termination. All other body weight changes are normal.

Gross pathology

Necropsy revealed abnormalities of the treated skin and thymus. Seven animals were normal at necropsy.

Other findings

Dermal Observations:Dermal effects ranged from absent to very slight on Day 1, absent to severe on Day 7 and absent to very slight on Day 14.

Applicant's summary and conclusion

Interpretation of results : not classified

Criteria used for interpretation of results

other: REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008

Conclusions

The dermal LD50 of Dimethyl Disulfide 94:5:1 is greater than 5000 mg/kg of body weight.

Executive summary

The acute dermal toxicity of Dimethyl Disulphide 94:5:1 was evaluated in rats according to OPPTS 870.1200 (August 1998) guideline. The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.Five male and female Wistar Albino rats were dosed dermally withDimethyl Disulfide 94:5:1 at 5000 mg/kg of body weight. The test article was kept in contact with the skin for 24 hours.Clinical signs, skin reactions, mortality and body weight gain were checked for a period of up to 14 days following the single administration. All animals were subjected to necropsy.

All ten animals survived the 5000 mg/kg dermal application.Instances of wetness and soiling of the anogenital area, chromorhinorrhea, sagging eyelids, emaciatedappearance, few feces, lethargy, ataxia, wet red substance on anogenital and nose/mouth areas were noted during the study.Dermal effects ranged from absent to very slight on Day 1, absent to severe on Day 7 and absent to veryslight on Day 14.Two animals lost weight from Day 0 to Day 7, but gained weight overall by study termination. All other bodyweight changes are normal. Necropsy revealed abnormalities of the treated skin and thymus. Seven animals were normal at necropsy. The dermal LD_0 of Dimethyl Disulfide 94:5:1 is greater than 5000 mg/kg of body weight.

[Study 2] Shapiro (PSL) 1986/K4 WoE /Acute dermal toxicity, rabbit

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	weight of evidence	4 (not assignable)	Shapiro R	1986	DIMETHYL DISULFIDE. EPA acute dermal|toxicity limit test.

Materials and methods

Test type : standard acute method

Limit test : yes

Test guideline

Qualifier	Guideline	Deviations
according to	EPA OPPTS 870.1200 (Acute Dermal Toxicity)	no

GLP compliance : yes

Test materials

DMDS tested in this study did not correspond to the specifications of DMDS proposed for registration (high level of methyl mercaptan).

Test animals

Species : rabbit

Strain : New Zealand White

Sex : male/female

Details on test animals and environmental conditions

TEST ORGANISMS:
- Source: Davidson's Mill Farm, S. Brunswick, NJ
- Age: no data
- Weight at study initiation: 2.4-2.7 kg for males, 2.1-2.3 kg for females
- Acclimatation period: 7 days
- Number of animals: 5 males + 5 females
- Controls: no

HOUSING
The animals were housed individually in wire bottomed cages
FOOD and WATER
- Food: Purina rabbit pellets, ad libitum
- Water: ad libitum

ENVIRONMENTAL CONDITIONS
- Temperature : 67-74°F
- Relative humidity : no data
- Light/dark cycle : no data
- Ventilation : no data

Administration / exposure

Type of coverage : occlusive

Vehicle : unchanged (no vehicle)

Details on dermal exposure

Five male and five female rabbits were prepared by clipping the skin free of hair over approximately 10% of the body surface (dorsal and ventral surface and side from scapular to pelvic area). Two g/kg body weight of the test material (applied neat) was placed over a 4-5 cm2 area on each rabbit. The patches were secured in place with ad-hesive tape and an elastic sleeve. Neck collars were then placed on each rabbit for a period of 24 hours. After 24 hours exposure to the test material, the patches were removed and the exposed surface was wiped clean of any residual test material using a damp cloth.

Duration of exposure : 24 hours

Doses : 2000 mg/kg

No. of animals per sex per dose : 5

Control animals : not required

Details on study design

The rabbits were observed for gross toxicity and mortality 5, 10, 30 min. and 1, 2, 4 h immediately after test material application, and at least once daily for a period of 14 days. Since there were no mortalities, gross necropsies were performed on all survivors at terminal sacrifice. The body weights of each rabbit wer recorded on the day of dosing and at 7 and 14 days.

Statistics

Not appropriate

<u>Results and discussions</u>

Effect levels

Sex	**Endpoint**	**Effect level**	**Based on**	**95% CL**	**Remarks**
male/female	LD0	>= 2000 mg/kg bw			Post exposure clinical signs: heavy breathing, loss of righting reflex, spontaneous spasms, pupillary dilation and constriction, unwillingness to stand, lethargy, excessive salivation and flared nostrils.

Mortality

None

Clinical signs

Within 5 minutes of test material application and for approximately 4 hours thereafter, the following symptoms were observed: heavy breathing, loss of righting reflex, spontaneous spasms, pupillary dilation and constriction, unwillingness to stand, lethargy, excessive salivation and flared nostrils. By 24 hours after dosing, the rabbits appeared active and healthy although skin discoloration and erythema was noted at the test sites. Eschar vas noted at the test sites of all rabbits on day 6 with sloughing tissue observed on day 9. All rabbits survived and were euthanized by intravenous injection of sodium pentabartitol on day 14.

Body weight

All rabbits gained weight over the 14 day observation period.

Gross pathology

Necropsy observations revealed dark foci on all lobes and surfaces of the lungs, pale purple or cloudy discoloration of the spleen accompanied by rough texture and edges in most rabbits. Slight hemorrhage, dark foci and indentation were

noted on the surface of the kidneys of a few animals.

Applicant's summary and conclusion

Interpretation of results : not classified

Criteria used for interpretation of results
other: REGULATION (EC) No 1272-2008

Conclusions
The acute dermal DL0 of Dimethyl Disulfide is > 2.0 g/kg body weight.

Executive summary
The acute dermal toxicity of DIMETHYL DISULFIDE (DMDS) was evaluated in rabbits according to EPA 40 CFR 163.81-2 guidelines. DMDS was applied to the skin of ten New Zealand white rabbits (five males and five females) at the dose-level of 2000 mg/kg under occlusive dressing for 24 hours. Animals were then observed during 14 days for mortality, clinical signs, effects on body weight and then necropsied.
No mortality was observed. Within 5 minutes of the application and for approximately 4 hours thereafter, heavy breathing, loss of righting reflex, spontaneous spasms, pupillary dilation and constriction, unwillingness to stand, lethargy, excessive salivation and flared nostrils were observed. By 24 hours after dosing, the rabbits appeared active and healthy although skin discoloration and erythema was noted at the test sites. Eschar vas noted at the test sites of all rabbits on day 6 with sloughing tissue observed on day 9. No effect on body weight was recorded. Necropsy observations revealed dark foci on all lobes and surfaces of the lungs, pale purple or cloudy discoloration of the spleen accompanied by rough texture and edges in most rabbits. Slight hemorrhage, dark foci and indentation were noted on the surface of the kidneys of a few animals.
Under the experimental conditions, the dermal LD0 of DMDS is higher than 2000 mg/kg in male/female rabbits.

[Study 3] Shapiro (PSL) 1985/K4 WoE/Acute dermal toxicity, rabbit

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
weight of evidence		4 (not assignable)	Shapiro R	1985	DIMETHYL DISULFIDE. EPA acute dermal|toxicity limit test

Materials and methods

Test type : standard acute method

Limit test : yes

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: EPA 40 CFR 163.81-2	no

GLP compliance : yes

Test materials

DMDS tested in this study did not correspond to the specifications of DMDS proposed for registration (high level of methyl mercaptan).

Test animals

Species : rabbit

Strain : New Zealand White

Sex : male/female

Details on test animals and environmental conditions

TEST ORGANISMS: - Source: Davidson's Mill Farm, S. Brunswick, NJ - Age: no data - Weight at study initiation: 2.2-2.9 kg - Adaptation period: at least 7 days HOUSING The animals were housed individually in wire bottomed cages FOOD and WATER - Food: Purina rabbit pellets, ad libitum - Water: ad libitum ENVIRONMENTAL CONDITIONS - Temperature : 68-72°F - Relative humidity : no data - Light/dark cycle : 12h/12h - Ventilation : no data

Administration / exposure

Type of coverage : occlusive

Vehicle : unchanged (no vehicle)

Details on dermal exposure

Following an adaptation period of at least 7 days, five male and five female rabbits were prepared by clipping the skin free of hair over approximately 10% of the body surface (dorsal and ventral surface and side from scapular to pelvic area). A non-permeable patch containing 2 g/kg body weight of the test material (applied neat) was placed over a 4-5 cm2 area on each rabbit. The patches were secured in place with adhesive tape and an elastic sleeve. Neck collars were then placed on each rabbit for a period of 24 hours. After 24 hours exposure to the test material, the patches were removed and the exposed surface was wiped clean of any residual test material using a damp cloth. The rabbits were observed for gross toxicity and mortality at least twice daily for a period of 14 days. Since there were no mortalities, gross necropsies were performed on all survivors at terminal sacrifice. The body weights of each rabbit were recorded on the day of dosing and at 7 and 17 days.

Duration of exposure : 24 hours

Doses : 2000 mg/kg

No. of animals per sex per dose : 5

Control animals : not required

Details on study design

- Duration of observation period following administration: 14 days - Frequency of observations: twice daily- Frequency of weighing: Day 0, 7 and 14- Necropsy of survivors performed: yes

Statistics

Not appropriate

Results and discussions

Effect levels

Sex	**Endpoint**	**Effect level**	**Based on**	**95% CL**	**Remarks**
male/female	LD0	>= 2000 mg/kg bw			Post-exposure clinical signs: none

Mortality

None

Clinical signs

All rabbits appeared active and healthy throughout the test period. There were no overt signs of gross toxicity nor was there any evidence of severe skin lesions.

Body weight

Eight rabbits gained weight over the 14 day observation period and two remained the same

Gross pathology

Gross necropsies were unrevealing. All organs and tissues appeared normal.

Applicant's summary and conclusion

Interpretation of results : not classified

Criteria used for interpretation of results:
EU

Conclusions

The acute dermal DL0 of Dimethyl Disulfide is > 2.0 g/kg body weight.

Executive summary

The acute dermal toxicity of DIMETHYL DISULFIDE (DMDS) was evaluated in rats according to EPA 40 CFR 163.81-2 guidelines. DMDS was applied to the skin of ten New Zealand white rabbits (five males and five females) at the dose-level of 2000 mg/kg under occlusive dressing for 24 hours. Animals were then observed during 14 days for mortality, clinical signs, effects on body weight and then necropsied.
No mortality was observed. No clinical signs nor effect on body weight was recorded. No gross pathology was found at necropsy.
Under the experimental conditions, the dermal LD0 of DMDS is higher than 2000 mg/kg in male/female rabbits.

[Study 4] Sheppard (Hazleton-IFT) 1985/K4 WoE/Acute dermal toxicity, rabbit

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
Study report		4 (not assignable)	Sheppard DB	1985	Détermination de la toxicité aiguë par voie percutanée chez le lapin

Materials and methods

Test type : standard acute method

Limit test : yes

Test guideline

Qualifier	Guideline	Deviations
according to	EU Method B.3 (Acute Toxicity (Dermal))	no

GLP compliance : no

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: no data

Test animals

Species : rabbit

Strain: New Zealand White

Sex : male/female

Details on test animals and environmental conditions

TEST ORGANISMS: - Source: Charles River, Saint Aubin-les-Elbeuf, France - Age: no data - Weight at study initiation: no data - Acclimatation period: no data HOUSING The animals were housed individually in wire bottomed cages FOOD and WATER - Food: UAR Formule 112, ad libitum - Water: ad libitum ENVIRONMENTAL CONDITIONS - Temperature : no data - Relative humidity : no data - Light/dark cycle : 12/12 h - Ventilation : no data

Administration / exposure

Vehicle : other: none

Details on dermal exposure

The test item was applied to the skin of one group of ten rabbits (five males and five females). The application was performed with the undiluted test item at the dose of 2000 mg/kg, under a non-permeable patch, over 10% of the body surface. The test site was then covered by a patch for 24 hours.

Duration of exposure : 24 hours

Doses : 2000 mg/kg

No. of animals per sex per dose : 5

Control animals : not required

Details on study design

Clinical signs, mortality and body weight gain were checked for a period of 15 days following the single application of the test item. All animals were subjected to necropsy.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
male/female	LD0	>= 2000 mg/kg bw			Post-exposure clinical signs: apathy and prostration

Mortality

No mortality was observed.

Clinical signs

Apathy and prostration were noted in most of the animals between 15 minutes and 3 hours after the application of the product. An increase in the spontaneous activity was noted for some animals the first day of treatment. The behavior of the animals during the remainder of the period of observation was considered normal.

Body weight

A very slight decrease of the boby weight was noted in some animals on D3. After, the body weight gain was considered satisfactory.

Gross pathology

No macroscopic lesion was observed at sacrifice.

Applicant's summary and conclusion

Interpretation of results : not classified

Criteria used for interpretation of results :
EU

Conclusions

The acute dermal DL0 of Dimethyl Disulfide is > 2.0 g/kg body weight.

[Study 5] Haynes (Toxicol) 1988/K4 WoE/Acute toxicity: dermal

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report		4 (not assignable)	Haynes G	1988	Acute Dermal Toxicity Study in the Rabbit

Materials and methods

Test type : standard acute method

Limit test : yes

Test guideline

Qualifier	Guideline	Deviations
according to	EPA OPP 81-2 (Acute Dermal Toxicity)	no

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: no data

Test animals

Species : rabbit

Strain : New Zealand White

Sex : male/female

Details on test animals and environmental conditions

Test animalsAge: Not reportedWeight at dosing: 2-3 kgSource: A. Smith, Warlingham, Surrey, England Acclimation period: 8 daysDiet: SQC standard rabbit pellets, SDS, Witham, Essex,England, ad libitumWater: Municipal water, ad libitumHousing: Grid bottom metal cagesEnvironmental conditions-Temperature: 17-23oCHumidity: 36-82%Air changes: Not specifiedPhotoperiod: 12 hours light/12 hours dark

Administration / exposure

Type of coverage : occlusive

Vehicle : unchanged (no vehicle)

Details on dermal exposure

One group of 5 males and 5 females was administered a single dose at 2000 mg/kg to clipped, intact skin for a period of 24-hours under an occlusive dressing.Individual doses of the test material were calculated based on body weights obtained just prior to dosing and taking into account the specific gravity of DMDS a dose level of 2000 mg/kg was achieved.On the day prior to dosing, the hair was removed from the backs of the animals using a small animal clipper. Individual doses of the test material were applied to the dorsal skin and covering approximately 10% of the total body area. Doses were applied under gauze binders that were secured with 10 cm wide ‘elastoplast’ adhesive elastic bandage. After 24 hours contact with the material the dressings were removed and the sites washed by gentle swabbing with cotton wool soaked in warm water.

Doses : 2000 mg/kg

No. of animals per sex per dose : 5

Control animals : no

Details on study design

All animals were examined frequently after dosing and then daily for 14 days in order to record time of onset and duration of any signs of toxicity observed. Body weights were recorded on days 1, 8, and 15 (termination). Upon termination, all animals were sacrificed by intravenous injection of sodium pentobarbitone. The major organ systems of the thoracic and abdominal cavities were examined for all animals.

Statistics

The data did not warrant statistical analysis.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
male/female	LD0	>= 2000 mg/kg bw			No mortality

Mortality

There was no mortality noted in this limit test for acute dermal toxicity.

Clinical signs

The following treatment related observations were recorded: constriction of the pupils in one male, iris inflammation in 2 females, nasal discharge in 3 males and 4 females and slow breathing in 2 males shortly after dosing. All animals had recovered within 1 hour of dosing.The test material induced skin irritation at the treatment site in all animals with discolouration also noted in 1 male following patch removal. The severity of this finding generally decreased with time and was replaced with desquamation and skin thickening.

Body weight

With the exception of 1 male that had a low weight gain (0.1 kg), the remaining animals gained weight (0.4 to 0.8 kg) within the normal limits over the 14 day observation period.

Gross pathology

One male had slightly mottled lungs and clear fluid in the thoracic cavity and 1 female also had slightly mottled lungs. In addition enlarged mesenteric lymph nodes were observed in 1 male. The significance of these findings was unclear.

Applicant's summary and conclusion

Interpretation of results : not classified

Criteria used for interpretation of results :
EU

Conclusions

The percutaneous LD50 of DMDS was greater than 2000 mg/kg for both males and females.

Executive summary

The acute dermal toxicity of dimethyl disulfide (DMDS) was evaluated in this limit test for acute dermal toxicity in the rabbit. The test material was applied once dermally at a dose of 2000 mg/kg to the clipped, intact skin of five male and five female rabbits for a 24-hour period under occlusive dressing. Mortality, clinical observations, dermal findings, body weights, and gross necropsy findings were evaluated on Day 1 (initiation) and for the next 14 days. There were no deaths following exposure of rabbits*via*the dermal route. Test material-related clinical observations of constriction of the pupils, inflammation of the iris or slow breathing were observed in 1 or 2 animals whilst nasal discharge was noted in 3 males and 4 females. Skin irritation at the treatment site was recorded in all animals following dosing and remained in some animals throughout the study. No findings were recorded at necropsy of the animals at the end of the observation period. Therefore it may be concluded that DMDS did not cause significant toxicity and there were no deaths following treatment at 2000 mg/kg*via*dermal application.

3.3 Acute toxicity - inhalation route

[Study 1] Kirkpatrick (WIL) 2005/K1 KS/Acute inhalation toxicity, 4h, rat

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable with restrictions)	Kirkpatrick DT	2005	Acute inhalation toxicity study study of dimethyl disulfide (DMDS) in albino rats

Materials and methods

Test type : standard acute method

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
according to	EPA OPPTS 870.1300 (Acute inhalation toxicity)	no

GLP compliance : yes (U.S. EPA Good Laboratory Practice Standards (40 CFR Part 160) and OECD Principles of Good Laboratory Practice)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.72%

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : male/female

Details on test animals and environmental conditions

TEST ORGANISMS:
- Source: Charles River Laboratories, Raleigh, North Carolina, USA
- Age: 8 to 12 weeks
- Weight at study initiation: 254 g to 307 g for males and from 226 g to 260 g for females
- Acclimatation: for a minimum of 7 days.

HOUSING
Individual suspended wire-mesh cages

FOOD and WATER
- Food: PMI Nutrition International, LLC, Certified Rodent LabDiet 5002, ad libitum
- Water: ad libitum municipal water, delivered by an automatic watering system

ENVIRONMENTAL CONDITIONS
- Temperature : 21.5°C to 21.9°C
- Relative humidity : 33.0% to 48.3%
- Light/dark cycle : 12h/12h
- Ventilation : filtered, not recycled air

Administration / exposure

Route of administration : inhalation: vapour

Type of inhalation exposur: whole body

Vehicle : other: unchanged (no vehicle)

Details on inhalation exposure

The acute inhalation toxicity of dimethyl disulfide (DMDS) was evaluated in a 4-hour, single-exposure study in rats. DMDS was initially administered to a single group of five male and five female Sprague-Dawley albino rats via whole-body vapor exposure at concentrations of 847, 1188, 1308 and 1650 ppm. Exposures were conducted in an approximately 165-L plexiglas whole-body exposure chamber, which allowed all animals to be easily seen and observed during exposure. Animals were housed individually during exposure. Food and water were withheld during the exposures. Exposure atmosphere conditions (temperature, relative humidity and oxygen content) were recorded approximately hourly during each exposure.

Analytical verification of test atmosphere concentrations

yes (Analyzed exposure concentrations were obtained using a GC. Samples of the exposure atmospheres were collected at least once every 30 minutes.)

Duration of exposure : 4 h

Concentrations : 847, 1188, 1308 and 1650 ppm (3.26, 4.57, 5.04 and 6.35 mg/L)

No. of animals per sex per dose : 5

Control animals : no

Details on study design

Mortality, clinical observations for pharmacotoxic signs and body weight changes were evaluated over a 14-day observation period. Detailed clinical observations (to determined peak effect of test article exposure) were conducted immediately following each exposure and at 1, 2, 4, 6 and 8 hours post-exposure. All animals were subjected to a gross necropsy.

Statistics

LC50 values (in ppm) and slopes (with 95% confidence limits) were calculated by the method of Litchfield and Wilcoxon.57,

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Exp. duration	Remarks
male/female	LC50	1310 ppm		1167 — 1471	4 h	(5.05 (4.49-5.66) mg/l) Clinical signs consisted of rales, decreased defecation and dried material on various body surfaces, including the mouth and nose.

Mortality

Concentration Mortality
847 ppm 0/10
1188 ppm 4/10
1308 ppm 4/10
1650 ppm 9/10
All deaths were noted during exposure or immediately following exposure

Clinical signs

Toxicologically relevant pharmacotoxic signs consisted of rales in the 1188 and 1650 ppm groups, decreased defecation in the 1188 ppm group and dried material on various body surfaces, including the mouth and nose for the surviving 1650 ppm female. Detailed clinical observations immediately following exposure consisted of salivation in the 1188, 1308 and 1650 ppm group males and/or females, tremors in the 1188, 1308 and 1650 ppm group females, low arousal in the 847, 1188 and 1308 ppm group females, ataxia and impaired mobility in the 1188 ppm group females, hunched gait in the 1308 and 1650 ppm group females, and increased difficulty in removing from cage/handling in females in all groups. Over the course of the 8-hour observation interval, females in all groups continued to have tremors, low arousal levels and were harder to remove from their cages and handle. Hunched gait was noted in the 1188 and/or 1308 ppm groups throughout the 8-hour period.

Body weight

Slight body weight losses (2 to 7 grams) were noted for one or more females each in the 847, 1188, 1308 ppm groups during the study. There were no other remarkable body weight changes. All surviving animals met or surpassed their initial (study day 0) body weight by study day 14.

Gross pathology

Dark red discoloration of the lungs was noted for animals found dead during or immediately following exposure in the 1308 and 1650 ppm groups. One male in the 1308 ppm group also was noted with lungs that did not fully collapse at necropsy. There were no gross findings for any of the found dead animals in the 1188 ppm group or for the surviving animals at the scheduled necropsy.

Any other information on results incl. tables

Total Incidence of Clinical Observations Immediately Following Exposure (Number of Occurrences/Number of Animals)

	DMDS (ppm)							
	Males				**Females**			
	847	**1188**	**1308**	**1650**	**847**	**1188**	**1308**	**1650**
Number of Rats	**5**	**2**	**3**	**0**	**5**	**4**	**3**	**1**
Salivation (slight)	-	1/2	1/3	-	-	-	1/3	-
Salivation (severe)	-	-	-	-	-	3/4	2/3	1/1
Tremors (slight - 1.5 mm)	-	-	-	-	1/5	-	1/3	-
Ease of Removal (moderately difficult)	-	-	-	-	-	-	1/3	-
Ease of Handling (moderately low)	-	-	-	-	4/5	4/4	3/3	1/1
Low Arousal	-	-	-	-	1/5	1/4	1/3	-
Hunched Gait	-	-	-	-	-	-	3/3	1/1
Ataxia	-	-	-	-	-	1/4	-	-

Impaired Mobility (moderately)	-	-	-	-	-	1/4	-	-

- = No observations at this interval.

Applicant's summary and conclusion

Interpretation of results : toxic (Category 3)

Criteria used for interpretation of results
other: REGULATION (EC) No 1272-2008

Conclusions
The LC50 of dimethyl disulfide was 1310 ppm (with 95% confidence limits of 1167-1471 ppm) when male and female albino rats were exposed whole-body to a vapor of the test article for a single, 4-hour period. The time of peak effect of DMDS was considered to be immediately following to 1 hour post-exposure.

Executive summary
The acute inhalation toxicity of dimethyl disulfide (DMDS) was evaluated in 4-hour, single-exposure study in rats performed in compliance with the EPA/OPPTS Guideline 870.1300 (1998) and the OECD Guidelines # 403 (1981). DMDS was initially administered to a single group of five male and five female Crl:CD(SD) albino rats via whole-body vapor exposure at concentrations of 847, 1188, 1308 and 1650 ppm. Mortality, clinical observations for pharmacotoxic signs and body weight changes were evaluated over a 14-day observation period. Detailed clinical observations (to determined peak effect of test article exposure) were conducted immediately following each exposure and at 1, 2, 4, 6 and 8 hours post-exposure. All animals were subjected to a gross necropsy.
Mortality was 0/0, 4/10, 4/10 and 9/10 animals for the 847, 1188, 1308 and 1650 ppm groups, respectively. All deaths were noted during exposure or immediately following exposure. Toxicologically relevant pharmacotoxic signs consisted of rales in the 1188 and 1650 ppm groups, decreased defecation in the 1188 ppm group and dried material on various body surfaces, including the mouth and nose for the surviving 1650 ppm female. Detailed clinical observations immediately following exposure consisted of salivation in the 1188, 1308 and 1650 ppm group males and/or females, tremors in the 847 and 1308 ppm group females, low arousal in the 847, 1188 and 1308 ppm group females, ataxia and impaired mobility in the 1188 ppm group females, hunched gait in the 1308 and 1650 ppm group females, and increased difficulty in removing from cage/handling in females in all groups. Over the course of the 8-hour observation interval, females in all groups had tremors, low arousal levels and were harder to remove from their cages and handle. Hunched gait was noted in the 1188 and/or 1308 ppm groups throughout the 8-hour period.
Slight body weight losses (2 to 7 grams) were noted for one or more females each in the 847, 1188, 1308 ppm groups during the study. There were no other remarkable body weight changes. All surviving animals met or surpassed their initial (study day 0) body weight by study day 14.
Dark red discoloration of the lungs was noted for animals found dead during or immediately following exposure in the 1308 and 1650 ppm groups. One male in the 1308 ppm group also was noted with lungs that did not fully collapse at necropsy. There were no gross findings for any of the found dead animals in the 1188 ppm group or for the surviving animals at the scheduled necropsy.
Based on the results of this study, the LC50 of dimethyl disulfide was 1310 ppm (with 95% confidence limits of 1167-1471 ppm) when male and female albino rats were exposed whole-body to a vapor of the test article for a single, 4-hour period. The time of peak effect of DMDS was considered to be immediately following to 1 hour post-exposure.

[Study 2] Kirkpatrick (WIL) 2008/K1 KS/1 and 5-d inhalation toxicity

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable with restrictions)	Kirkpatrick DT	2008	A 5-day inhalation toxicity study of dimethyl disulfide (DMDS) in albino rats with microscopic examination of the upper respiratory tract

study report	Key study	1 (reliable with restrictions)	Haber L, Parker A and Dourson M	2008	Benchmark dose (BMD) and Categorical Regression Modeling of Acute Exposure to Dimethyl Disulfide (DMDS)

Materials and methods

Test type : other: A 5-day inhalation toxicity study with microscopic examination of the upper respiratory tract

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		

Principles of method if other than guideline

Evaluation of the potential toxic effects of dimethyl disulphide (DMDS) on tissues of the upper respiratory tract (URT) when administered 6 hours/day as a vapor via whole-body inhalation to rats for 1 day or 5 consecutive days.

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.9%

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Laboratories, Inc., Raleigh, North Carolina, USA
- Age at study initiation: 8-9 weeks old
- Weight at study initiation: 240 g to 305 g for males and from 178 g to 234 g for females.
- Fasting period before study: none
- Housing: individually in clean, stainless steel, wire-mesh cages suspended above cage-board.
- Diet (ad libitum): PMI Nutrition International, LLC, Certified Rodent
- Water (ad libitum): reverse osmosis-treated (on-site) drinking water,
- Acclimation period: at least 9 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 21.2 to 21.8
- Humidity (%): 38.3 to 45.8
- Air changes (per hr): 10
- Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration : inhalation: vapour

Type of inhalation exposure : whole body

Vehicle : other: unchanged (no vehicle)

Details on inhalation exposure

GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION
- Exposure apparatus: stainless-steel and glass whole-body exposure chambers
- Exposure chamber volume: 1000 L
- Method of holding animals in test chamber: animals were individually housed in stainless steel wire-mesh caging suspended over cageboard during the exposures.
- Source and rate of air: HEPA-and charcoal-filtered air at a flow of 12-15 air changes per hour.
- Method of conditioning air:
- System of generating vapors: vapors of DMDS were generated by bubbling metered nitrogen through gas-washing bottles containing appropriate aliquots of the test article. Prior to entering the test chambers, the vapors of the test article were mixed with dilution air to achieve the desired exposure levels.
- Treatment of exhaust air:
- Temperature, humidity, pressure in air chamber: 20°C and 24°C, 30% to 70%, approximately 12 to 15 air changes per hour (200 to 250 LPM) at a slight negative pressure.
TEST ATMOSPHERE
- Brief description of analytical method used: analyzed concentrations of the test article in each exposure chamber were determined by a specific on-line gas chromatographic method and were measured approximately every 35 minutes throughout the exposure period. The calibration of the gas chromatograph was considered acceptable if the r2 value for the prime calibration curve was ≥ 0.98 and the individual points of the calibration werewithin 10% of their target concentrations.
- Samples taken from breathing zone: no

Analytical verification of test atmosphere concentrations : yes

Duration of exposure : 6 h

Concentrations : 50, 150, 300 and 600 ppm (192, 577, 1154 and 2318 mg/m3)

No. of animals per sex per dose

The control group (Group 1) and Groups 2-5 each consisted of 20 animals/sex.Following one 6-hour exposure, 10 animals/sex/group were euthanized (approximately 24 hours following the single exposure); the remaining 10 animals/sex/group were exposed for an additional 4 days (total of 5 consecutive exposure days) and were euthanized approximately 24 hours following the final exposure.

Control animals : yes

Details on study design

CLINICAL OBSERVATIONS AND SURVIVAL
All animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity.During the exposure phase, clinical examinations were performed twice daily, prior to and following each exposure.
BODY WEIGHTS
Individual body weights were recorded during the pretest period, beginning approximately 1 week prior to test article exposure (study day -7 for males) and prior to the first, third and fifth exposures (study days 0, 2, and 4, respectively). Final body weights (fasted) were recorded prior to the scheduled necropsies.
MACROSCOPIC EXAMINATION
A complete necropsy was conducted on all animals at the scheduled necropsies.The following tissues and organs were collected and placed in 10% neutral-buffered formalin (except as noted):Kidneys (2)LarynxLiver (sections of 2 lobes)Lungs (including bronchi, fixed by inflation with fixative)Nasal tissues*TracheaGross lesions (when possible)* = Following fixation in formalin, nasal tissues were decalcified and sections of 6 nasal levels were prepared for microscopic examination.
ORGAN WEIGHTS
The following organs were weighed from all animals at the scheduled necropsies: kidneys, liver and lungs (prior to inflation with fixative)
MICROSCOPIC EXAMINATION
Microscopic examination was performed on nasal tissues and gross lesions from all animals in all exposure groups. Nasal sectioning was performed using the method of Morgan in which 6 nasal sections were produced using specifically defined landmarks for sectioning (Mery et al., 1994b). All 6 nasal levels were examined microscopically.

Statistics

Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test article-treated group to the control group by sex.Body weight, body weight change and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to

determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test article-treated groups to the control group.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Exp. duration	Remarks
male/female	other: BMD10% for nasal irritation (severity 2)	19 ppm		9.3 — 28.7	6 h	70 (53 - 110) mg/m3. Use of severity 2 would be consistent with a determination that minimal effects are not adverse.

Mortality

All animals survived to the scheduled necropsies.

Clinical signs

There were no test article-related clinical observations.

Body weight

Test article-related effects on body weight and/or body weight gain were noted for males and females at all exposure levels. During study days 0-2, lower mean body weight gains or mean body weight losses were noted in the 50, 150, 300 and 600 ppm group males and females; the differences from the control group were generally statistically significant ($p<0.01$). During study days 2-4, mean body weight gains in these same groups were significantly ($p<0.01$) lower for the males but similar to the control group values for the females. As a result of the body weight gain deficits, mean body weights in the 50, 150, 300 and 600 ppm group males on study days 2 and 4 were significantly ($p<0.05$ or $p<0.01$) lower than the control group values; the differences on study day 4 (8.8% to 15.4% lower) were greater than those noted for study day 2. Mean female body weights on study days 2 and 4 were similar to (50 and 150 ppm groups) or lower than (300 and 600 ppm groups) the control group values; the differences at 300 and 600 ppm (6.7% to 8.1% lower) were generally significant ($p<0.01$ or $p<0.05$).There were no test article-related changes in mean final body or organ weights at the interim necropsy.At the primary necropsy, mean final body weights were statistically significantly ($p<0.01$) lower in all test article-exposed male groups. These mean body weight changes in males showed a dose relationship at the highest 2 exposure levels (differences of 9.5%, 9.5%, 10.2% and 16.4% in the 50, 150, 300 and 600 ppm groups, respectively, compared to the control group mean) and were considered test article-related in all groups. Mean final body weights in test article-exposed female groups were not significantly different from the control group mean.

Gross pathology

There were no test article-related macroscopic findings at the scheduled necropsies

Other findings

- Organ weights: In females at the primary necropsy, mean absolute lung and mean lung/body weights were higher in the 300 ppm group (7.8% and 14.9%, respectively) and the 600 ppm group (21.6% and 29.1%, respectively) when compared to the control group values. Except for the mean absolute lung weight in the 300 ppm group, these changes were statistically significant ($p<0.01$) and were considered test article-related. In males, the mean lung/final body weights were statistically significantly ($p<0.01$) higher in the 300 and 600 ppm groups (15.6% and 23.8%, respectively), but the mean absolute lung weights in these groups were similar to the control group. The relationship of lung/final body weight changes in males to test article exposure cannot be determined because the changes were potentially confounded by significantly lower mean final body weights in the test article-exposed male groups.Other statistically significant organ weight differences were observed in males at the primary necropsy but were considered secondary to lower mean final body weights; these differences were lower mean kidney weights in the 150 and 600 ppm groups, higher mean kidney/final body weight in the 600 ppm group and lower mean liver weights in all test article-exposed groups. In females, statistically significantly higher mean kidney/body weight for the 600 ppm group at the primary necropsy was considered secondary to slightly lower but not statistically significant mean body weight in thatgroup, since the mean absolute kidney weight for the 600 ppm group females was not different than that of the control group.
- Histopathology: (for tables see the attached document)

INTERIM NECROPSY (1-DAY EXPOSURE)

After a single 6-hour whole-body exposure to dimethyl disulfide at concentrations of 50, 150, 300 or 600 ppm, test article-related changes were observed in 3 types of epithelia (transitional, respiratory and olfactory epithelia) and in all 6 levels; changes in squamous epithelium were suggestive of an effect of exposure. Olfactory epithelium, which was consistently present in Levels III-VI, and respiratory epithelium, which was consistently present in Levels II-VI, showed decreasing lesion severity with progressively more posterior levels. Transitional epithelium, which was consistently present only in the more anterior nasal levels (Levels I and II), generally did not show an anterior-posterior gradient of lesion severity. Degenerative changes at a particular level, except for Level I, were generally dose-related in incidence and/or severity.Minimal acute inflammation of the squamous epithelium was observed in Level I in all test article-exposed groups and was characterized by focal to multifocal infiltrates of primarily eosinophils and neutrophils in the mucosa/submucosa, sometimes accompanied by mucosal edema, thinning, erosion and/or ulceration. Incidences are shown in Text Table 3. The incidences of this finding were not related to exposure concentration, but because the inflammation was observed only in test article-exposed groups, it was considered a probable effect of test article exposure. No test article exposure-related changes in squamous epithelium were noted in Nasal Level II.Test article exposure-related changes in the transitional epithelium were observed in Nasal Levels I and II and are presented in Text Table 4. Degeneration of the transitional epithelium was characterized by epithelial cell flattening, vacuolation, individualization, pyknosis, erosion and/or ulceration and was observed in all test article-exposed groups in both Nasal Levels I and II. The severity of degeneration was dose-related in Nasal Level II. In Nasal Level I, the incidences and severities were dose-related in females but not in males, possibly because of the inconsistent presence of transitional epithelium in this level.Inflammation of the transitional epithelium was also observed in Levels I and II, and was characterized by edema and polymorphonuclear infiltrates within the mucosa and/or submucosa, with occasional polymorphonuclear luminal exudates when ulcers were present. In Nasal Level II, all animals from the 150 ppm and higher groups, as well as most males and half of the females from the 50 ppm group, were affected. The severity of acute inflammation was dose-related in males in Level II, while in females in Level II, the severity was higher in the 150 ppm and higher exposure groups than in the 50 ppm group but did not show a clear dose response. In Level I, all test article-exposed groups except males exposed to 150 ppm were affected, but there was no dose response in incidence or severity, possibly because of the inconsistent presence of transitional epithelium in this level, as previously mentioned. Test article exposure-related changes in the respiratory epithelium were observed in all 6 nasal levels and are presented in Text Table 5.Degeneration of the respiratory epithelium was characterized by flattening, loss of cilia, vacuolation and/or pyknosis of the epithelial cells. Mildl degeneration was observed in a few of the 300 and 600 ppm group males and 600 ppm group females in Nasal Level I. Degeneration was observed most frequently in Levels II and III at higher exposure concentrations, with the highest incidence and severity at 600 ppm in these levels. The incidence and severity of respiratory epithelial degeneration decreased in the more caudal levels; in Level IV it was only observed at 600 ppm, and it was not observed at all in Levels V or VI.An increased incidence of acute inflammation of the respiratory epithelium was noted in males and females in at least 1 nasal level in all test article exposure groups. The test article exposure groups affected varied with the nasal level. The incidence and severity of inflammation at a given exposure concentration of test article generally decreased in nasal levels caudal to Nasal Level III. Additionally, inflammation was observed farther caudally at higher exposure concentrations. For instance, in males, acute inflammation of the respiratory epithelium was observed in Level II at 50 ppm, in Levels II and III at 150 ppm, and in Levels II-IV at 300 and 600 ppm. Incidences of inflammation in male Nasal Levels V and VI were similar to control incidences. In females, respiratory epithelial inflammation extended to Nasal Level V at 150, 300 and 600 ppm and to Nasal Level VI at 600 ppm.Test article exposure-related degeneration and/or inflammation of the olfactory epithelium were observed in Nasal Levels II-VI, as presented in Text Tables 6 and 7. Degeneration of the olfactory epithelium was characterized by sloughing of sensory and sustentacular cells that resulted in only a thin layer of basal cells remaining. Less severe degeneration was noted as focal or multifocal pyknosis and/or vacuolation of sensory epithelium resulting in an irregular surface and decreased nuclear density from sensory cell loss. Bowman's glands and nerve bundles in the lamina propria were not affected at any test article exposure concentration. At all levels except Level II, olfactory epithelial degeneration showed a dose-response relationship in incidence and/or severity at all test article exposure concentrations. Additionally, at a particular exposure, the incidence and/or severity of degeneration generally decreased with progressively more caudal sections. Higher exposure concentrations generally caused more degeneration farthercaudally than lower exposure concentrations. Acute inflammation of the olfactory epithelium, observed in Levels II-VI, was noted in at least 1 nasal level at all test article exposure levels. Inflammation generally showed a dose response in incidence and/or severity with higher exposure concentration for a given nasal level, and occurred with lower incidence and severity with progressively farther caudal sections at a given exposure concentration.

PRIMARY NECROPSY (5-DAY EXPOSURE)

After 5 consecutive days of 6-hour/day whole-body exposure to dimethyl disulfide at concentrations of 50, 150, 300 or 600 ppm, test article-related changes were observed in all 4 epithelial cell types and in all 6 nasal levels. As at the interim necropsy, olfactory and respiratory epithelia showed decreasing incidence and/or severity of degeneration and inflammation with progressively more caudal levels at a given exposure concentration. Squamous and transitional epithelia generally did not show an anteriorposterior gradient of lesion severity. All 4 types of epithelia showed

evidence of regeneration/hyperplasia despite continued exposure to the test article, with an anteriorposterior gradient of incidence/severity for olfactory and respiratory epithelia. Changes in squamous epithelium were observed in Levels I and II and are presented in Text Table 8.Minimal to mild acute inflammation of the squamous epithelium was observed sporadically in Nasal Level I. At this level, the highest incidence in both males and females was in the 300 ppm exposure group. Despite the lack of a dose response, inflammation was considered to be test article-related because it was not observed in the control group.Hyperplasia of the squamous epithelium was observed as thickening of the epithelial cell layer. In females, it was noted in all test article-exposed groups in Nasal Level I. In males, squamous epithelial hyperplasia was limited to the 300 and 600 ppm exposure concentrations in Nasal Levels I and II. There was a general increase in incidence/severity with exposure concentration except at the highest exposure level in females.Test article exposure-related changes in the transitional epithelium were observed in Nasal Levels I and II and are presented in Text Table 9.Test article-related changes in the transitional epithelium consisted of degeneration, hyperplasia and inflammation. Degeneration was observed in all test article-exposed male and female groups in Nasal Level II. At this level, compared to the study day 1 necropsy, the overall severity of degeneration was decreased for all test article-exposed groups and the incidences of degeneration were decreased for the 50 and 150 ppm groups, possibly because of the presence of transitional epithelial hyperplasia at study day 5.Hyperplasia of the transitional epithelium was characterized by increased thickness of the epithelial layer, nuclear disorganization, and/or loss of apical cytoplasm, often with flattening of the adluminal cells. Hyperplasia was observed in all test article exposure groups in Nasal Level II, but was less consistent in Nasal Level I, possibly because transitional epithelium was inconsistently present in that level.Inflammation of the transitional epithelium was also observed in Nasal Levels I and II. The incidence in all test article-exposed female groups and 150, 300 and 600 ppm male groups was increased compared to controls in at least 1 level. Inflammation was most consistently observed in Nasal Level II. At the primary necropsy, the severity of transitional epithelial inflammation was generally higher in the more caudal level (Level 2) compared to the interim necropsy for all exposure concentrations. One 300 ppm group male had osteitis of the nasoturbinate in Nasal Level II that resulted from inflammation and ulceration of the overlying transitional epithelium and exposure of the bone.Test article exposure-related changes in the respiratory epithelium after 5 consecutive days of whole body exposure to DMDS consisted of degeneration, hyperplasia and acute inflammation. Incidences of selected changes are presented in Text Table 10.Unlike at the interim necropsy, in which respiratory epithelial degeneration was observed at 50 ppm and higher exposure concentrations, at the primary necropsy respiratory epithelial degeneration was observed only at the 600 ppm exposure concentration. There were no consistent changes in the incidences or severities of degeneration at 600 ppm when the interim and primary necropsies were compared, although degeneration was observed farther caudal (in Nasal Level V) in females at study day 5 compared to study day 1. The apparent lack of degeneration at lower exposure concentrations at the primary necropsy may be secondary to the presence of respiratory epithelial hyperplasia.Hyperplasia of the respiratory epithelium was characterized by increased thickness of the epithelial layer, nuclear disorganization, and/or loss of cilia and apical cytoplasm, often with flattening of the adluminal cells. Hyperplasia was inconsistent in Nasal Level II, possibly because of the variable presence of respiratory vs. transitional epithelium in this level. Hyperplasia was consistently observed in Nasal Level III at exposure concentrations of 50 ppm and higher, and generally showed a dose response relationship in incidence and/or severity in this nasal level. Respiratory epithelial hyperplasia was not observed caudal to Nasal Level III at 50 ppm and was observed in Nasal Level IV, but not Levels V or VI, at 150 ppm. At the two highest exposure levels, respiratory epithelial hyperplasia extended to Nasal Level VI, although the incidence and severity decreased in the 300 ppm exposure concentration group in these caudal levels.Acute inflammation of the respiratory epithelium was observed at the 300 ppm and higher exposure concentrations in males and the 150 ppm and higher exposure concentrations in females; findings are presented in Text Table 11, below. There was a gradient of higher to lower incidences of inflammation with more caudal sections at a given exposure concentration. When compared to the 1-day exposure groups, test article exposure-related inflammation in the 5-day exposure groups was observed only at higher exposure concentrations for a given nasal level, but generally occurred at similar incidences and severities at these concentrations.Test article exposure-related degeneration, regeneration and/or inflammation of the olfactory epithelium were observed in Nasal Levels II-VI; findings are presented in Text Tables 12, 13 and 14. Whole body exposure to the test article for 5 days resulted in olfactory epithelial degeneration in Nasal Levels II-VI at all exposure concentrations.At 50 ppm, the incidences of degeneration were higher after 5 days of exposure in Nasal Levels II and III in males and in all nasal levels in females. Also in females, degeneration was observed farther caudal in the 50 and 150 ppm 5-day exposure concentrations than was observed at these concentrations after the single exposure. For both males and females, the severity of degeneration in the more anterior nasal levels (Nasal Levels II, III and IV) was generally less severe for a given exposure concentration after 5 days of exposure than after a single day exposure, primarily because of olfactory epithelial regeneration.Minimal to mild olfactory epithelial regeneration was present with degeneration in all test article-exposed groups of males and females in Nasal Levels II-VI. Regeneration consisted of areas of 1 or 2 layers of disorganized basophilic cells overlying the previously desquamated basal cell layer. Areas of regeneration were sometimes observed adjacent to areas of acute degeneration. Incidences of regeneration in Nasal Levels II-V were >80% and frequently near 100% for exposure concentrations of 150 ppm and higher, and decreased in incidence and severity in Nasal Level VI. At 50 ppm, incidences of olfactory epithelial regeneration became progressively lower caudal to Nasal Level III, but

extended to Nasal Level VI.Acute inflammation of the olfactory epithelium was observed in Levels III-VI at similar incidences and severities as were noted at the study day 1 interim necropsy. At a given nasal level and exposure concentration, there were no consistent differences in the incidence or severity of inflammation between animals exposed to DMDS for 1 or 5 days. As at the interim necropsy, the incidence of inflammation of the olfactory epithelium tended to increase with higher exposure concentration at a given nasal level, and occurred with lower incidence and severity in the caudal nasal levels at a given exposure; findings are presented in Text Table 14.In addition to changes in epithelia, whole-body exposure to DMDS for 5 consecutive days resulted in changes in the bony trabeculae of the nasal turbinates. Fibro-osseous proliferation of the bony trabeculae of the naso-, maxillo-, and/or ethmo-turbinates in Nasal Levels II-IV was observed in at least 1 nasal level in the 150 ppm and higher group males and 300 ppm and higher group females. Fibro-osseous proliferation was characterized by the presence of lightly basophilic new bone and increased fibroblast-like cells near the periosteal surface; findings are presented in Text Table 15. The incidence and severity of this lesion in Nasal Level II were related to exposure concentration, and showed an anterior-posterior decrease at a given exposure concentration.There were no other test article-related microscopic changes observed. All other microscopic observations were considered spontaneous or incidental, with no relationship to text article exposure.- Potential target organs: nasal epithelium

<u>Overall remarks, attachments</u>

Remarks on results including tables and figures

Toxicology Excellence for Risk Assessment (TERA) has conducted a benchmark dose/concentration (BMD/C) modeling and categorical regression modeling on the toxicology data derived from this 1- and 5-day inhalation exposure of male and female rats to dimethyl disulphide (DMDS). The results from TERA's benchmark concentration (BMC) and categorical regression modeling show that Region II is more sensitive than either Regions III or IV. Modeling results also show that the concentrationresponse slopes are quite steep. Initial modeling was conducted using BMC modeling, but satisfactory fit could not be obtained for the most sensitive region, Region II, for modeling of males only or both sexes combined.
Therefore, additional modeling was conducted using categorical regression modeling, which can model the concentration-severity-response, and uses more of the data. The modeling results were evaluated to identify appropriate points of departure for development of a 1-day risk assessment value. Several options are briefly discussed, yielding Extra Risk Concentrations (ERC's) of between 11 and 28 ppm, with lower bounds of between 4.8 and 15 ppm. The option 2 was selected for the purpose to derive an acute DNEL for local irritation (nasal irritation).

Options	ERC (& lower limit) in ppm	Considerations
Option 1 - Severity 1, ERC 10%, logit model, both sexes	11 (4.8)	Combination of sexes is statistically supp considerations support this combination. S minimal effects.
Option 2 - Severity 2, ERC 10%, logit model, both sexes	19 (9.3)	Use of severity 2 would be consistent with minimal effects are not adverse.
Option 3 - Severity 1, ERC 20%, logit model, both sexes	19 (9.7)	Severity includes very minimal effects, and severity, but incidence is higher than the resp categorical regression.
Option 4 - Severity 2, ERC 20%, logit model, both sexes	28 (15)	Use of points of departure higher than 10% allowed under EPA's BMC guidelines, but procedure.

As noted, the sexes have been combined for all options in this Table.

<u>INTERIM NECROPSY (1-DAY EXPOSURE)</u>

Text Table 3. Incidences of Acute Inflammation in Squamous Epithelium, Study Day 1 Interim Necropsy

	Males					Females				
Target Exposure Level (ppm):										
	0	**50**	**150**	**300**	**600**	**0**	**50**	**150**	**300**	**600**

Squamous epithelium, acute inflammation										
Nasal Level I[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Minimal	0	2	2	4	2	0	1	2	3	1

[a] = Number of tissues examined from each group.

Text Table 4. Incidences of Changes in Transitional Epithelium, Study Day 1 Interim Necropsy

	Males					**Females**				
Target Exposure Level (ppm):	**0**	**50**	**150**	**300**	**600**	**0**	**50**	**150**	**300**	**600**
Transitional epithelium, degeneration										
Nasal Level I[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Total incidence	0	1	1	4	1	0	1	3	8	9
Minimal	0	1	1	2	0	0	1	3	4	3
Mild	0	0	0	1	0	0	0	0	4	4
Moderate	0	0	0	1	1	0	0	0	0	1
Severe	0	0	0	0	0	0	0	0	0	1
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Total incidence	0	9	10	10	0	0	3	10	10	10
Minimal	0	7	2	0	0	0	3	0	0	0
mild	0	2	2	0	0	0	0	2	0	0
Moderate	0	0	3	3	0	0	0	4	5	2
Severe	0	0	3	7	10	0	0	4	5	8
Transitional epithelium, acute inflammation										
Nasal Level I[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Total incidence	0	2	0	4	1	0	6	2	8	9
Minimal	0	2	0	4	1	0	6	0	7	6
Mild	0	0	0	0	0	0	0	2	1	3
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Total incidence	3	9	10	10	10	0	5	10	10	10
Minimal	3	9	7	4	0	0	5	6	9	6
Mild	0	0	3	6	10	0	0	4	1	4

[a] = Number of tissues examined from each group.

Text Table 5. Incidences of Changes in Respiratory Epithelium, Study Day 1 Interim Necropsy

	Males					**Females**				
Target Exposure Level (ppm):	**0**	**50**	**150**	**300**	**600**	**0**	**50**	**150**	**300**	**600**
Respiratory epithelium, degeneration										
Nasal Level I[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence (%)	0 (0)	0 (0)	0 (0)	2 (20)	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)	3 (30)
mild	0	0	0	2	1	0	0	0	0	3
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence (%)	0 (0)	0 (0)	1 (10)	2 (20)	7 (70)	0 (0)	1 (10)	0 (0)	1 (10)	3 (30)
minimal	0	0	1	2	6	0	1	0	1	3
mild	0	0	0	0	1	0	0	0	0	0
Nasal Level III[a]	**10**	**7**	**8**	**7**	**10**	**9**	**9**	**8**	**8**	**8**
total incidence (%)	0 (0)	0 (0)	1 (13)	1 (14)	5 (50)	0 (0)	0 (0)	1 (13)	0 (0)	5 (63)
minimal	0	0	1	0	1	0	0	1	0	3
mild	0	0	0	1	4	0	0	0	0	2
Nasal Level IV[a]	**8**	**6**	**5**	**6**	**6**	**5**	**2**	**7**	**7**	**6**
total incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)	2 (33)	0 (0)	0 (0)	0 (0)	0 (0)	3 (50)
minimal	0	0	0	0	2	0	0	0	0	3
Respiratory epithelium, acute inflammation										
Nasal Level I[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence (%)	1 (10)	0 (0)	0 (0)	2 (20)	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
minimal	2	0	0	2	1	0	0	0	0	0
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence (%)	3 (30)	6 (60)	8 (80)	8 (80)	10 (100)	0 (0)	2 (20)	6 (60)	7 (70)	4 (40)
minimal	3	5	8	6	8	0	2	6	7	4
mild	0	1	0	2	2	0	0	0	0	0
Nasal Level III[a]	**10**	**7**	**8**	**7**	**10**	**9**	**9**	**8**	**8**	**8**
total incidence (%)	1 (10)	0 (0)	5 (63)	6 (86)	7 (70)	0 (0)	0 (0)	2 (25)	6 (75)	7 (88)
minimal	1	0	5	6	5	0	0	2	6	6
mild	0	0	0	0	2	0	0	0	0	1
Nasal Level IV[a]	**8**	**6**	**5**	**6**	**6**	**5**	**2**	**7**	**7**	**6**
total incidence (%)	3 (38)	0 (0)	2 (40)	5 (83)	5 (83)	0 (0)	1 (50)	3 (43)	4 (57)	6 (100)
minimal	3	0	2	5	4	0	1	3	4	5
mild	0	0	0	0	1	0	0	0	0	1
Nasal Level V[a]	**9**	**8**	**10**	**8**	**10**	**8**	**10**	**10**	**10**	**10**
total incidence (%)	2 (22)	0 (0)	1 (10)	2 (25)	3 (30)	0 (0)	0 (0)	1 (10)	1 (10)	4 (40)
minimal	2	0	1	2	3	0	0	1	1	4
Nasal Level VI[a]	**9**	**10**	**9**	**10**	**9**	**9**	**10**	**9**	**10**	**9**
total incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (11)
minimal	0	0	0	0	0	0	0	0	0	1

[a] = Number of tissues examined from each group.

Text Table 6. Incidences of Degeneration of Olfactory Epithelium, Study Day 1 Interim Necropsy

	Males					**Females**				
Target Exposure Level (ppm):	**0**	**50**	**150**	**300**	**600**	**0**	**50**	**150**	**300**	**600**
Olfactory epithelium, degeneration										
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence	0	6	10	9	10	0	2	10	10	9
minimal	0	3	1	0	2	0	0	1	0	1
mild	0	2	1	1	0	0	0	1	0	0
moderate	0	1	1	3	4	0	1	1	1	1
severe	0	0	7	5	4	0	1	7	9	7
Nasal Level III[a]	**10**	**7**	**8**	**7**	**10**	**9**	**9**	**8**	**8**	**8**
total incidence	0	6	8	7	10	0	2	8	8	8
minimal	0	1	0	0	0	0	1	1	0	0
mild	0	4	1	0	0	0	1	3	1	0
moderate	0	1	6	3	4	0	0	4	7	5
severe	0	0	1	4	6	0	0	0	0	3
Nasal Level IV[a]	**8**	**6**	**5**	**6**	**6**	**5**	**2**	**7**	**7**	**6**
total incidence	0	4	5	6	6	0	1	7	7	6
minimal	0	4	2	0	0	0	0	4	0	0
mild	0	0	2	4	0	0	1	3	3	1
moderate	0	0	1	2	4	0	0	0	4	4
severe	0	0	0	0	2	0	0	0	0	1
Nasal Level V[a]	**9**	**8**	**10**	**8**	**10**	**8**	**10**	**10**	**10**	**10**
total incidence	0	5	8	8	10	0	0	10	10	10
minimal	0	5	8	5	0	0	0	10	3	0
mild	0	0	0	3	5	0	0	0	7	3
moderate	0	0	0	0	5	0	0	0	0	6
severe	0	0	0	0	0	0	0	0	0	1
Nasal Level VI[a]	**9**	**10**	**9**	**10**	**9**	**9**	**10**	**9**	**10**	**9**
total incidence	0	2	4	9	9	0	0	0	3	7
minimal	0	2	4	5	4	0	0	0	3	3
mild	0	0	0	4	4	0	0	0	0	3
moderate	0	0	0	0	1	0	0	0	0	1

[a] = Number of tissues examined from each group

Text Table 7. Incidences of Acute Inflammation of Olfactory Epithelium, Study Day 1 Interim Necropsy

	Males					Females				
Target Exposure Level (ppm):	**0**	**50**	**150**	**300**	**600**	**0**	**50**	**150**	**300**	**600**
Olfactory epithelium, acute inflammation										
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence	0	1	5	1	3	0	1	2	4	2
minimal	0	1	5	1	3	0	1	2	4	2
Nasal Level III[a]	**10**	**7**	**8**	**7**	**10**	**9**	**9**	**8**	**8**	**8**
total incidence	0	1	4	5	8	0	0	2	8	6
minimal	0	1	3	4	6	0	0	2	8	6
mild	0	0	1	1	2	0	0	0	0	0
Nasal Level IV[a]	**8**	**6**	**5**	**6**	**6**	**5**	**2**	**7**	**7**	**6**
total incidence	0	0	3	4	6	0	0	2	7	6
minimal	0	0	3	3	4	0	0	2	7	6
mild	0	0	0	1	2	0	0	0	0	0
Nasal Level V[a]	**9**	**8**	**10**	**8**	**10**	**8**	**10**	**10**	**10**	**10**
total incidence	0	0	0	3	7	0	0	0	3	4
minimal	0	0	0	3	6	0	0	0	3	4
mild	0	0	0	0	1	0	0	0	0	0
Nasal Level VI[a]	**9**	**10**	**9**	**10**	**9**	**9**	**10**	**9**	**10**	**9**
total incidence	0	0	0	0	1	0	0	0	0	0
minimal	0	0	0	0	1	0	0	0	0	0

[a] = Number of tissues examined from each group.

Primary necropsy (5-day exposure)

Text Table 8. Incidences of Changes in Squamous Epithelium, Study Day 5 Primary Necropsy

	Males					**Females**				
Target Exposure Level (ppm):	**0**	**50**	**150**	**300**	**600**	**0**	**50**	**150**	**300**	**600**
Squamous epithelium, acute inflammation										
Nasal Level I[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence	0	1	0	4	1	0	0	0	6	1
minimal	0	1	0	4	1	0	0	0	4	1
mild	0	0	0	0	0	0	0	0	2	0
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence	0	0	0	0	1	0	0	0	0	0
minimal	0	0	0	0	1	0	0	0	0	0
Squamous epithelium, hyperplasia										
Nasal Level I[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence	0	0	0	5	1	0	2	6	9	5
minimal	0	0	0	3	1	0	2	6	3	3
mild	0	0	0	2	0	0	0	0	6	2
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence	0	0	0	1	2	0	0	0	0	0
minimal	0	0	0	0	1	0	0	0	0	0
mild	0	0	0	1	1	0	0	0	0	0

[a] = Number of tissues examined from each group.

Text Table 9. Incidences of Changes in Transitional Epithelium, Study Day 5 Primary Necropsy

	Males					Females				
Target Exposure Level (ppm):	**0**	**50**	**150**	**300**	**600**	**0**	**50**	**150**	**300**	**600**
Transitional epithelium, degeneration										
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence	0	4	2	10	9	0	3	1	10	10
minimal	0	4	2	3	3	0	2	1	5	2
mild	0	0	0	7	6	0	1	0	5	8
Transitional epithelium, acute inflammation										
Nasal Level I[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence	0	0	0	2	1	0	1	0	1	1
minimal	0	0	0	2	1	0	1	0	1	1
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence	3	3	7	9	9	0	2	5	10	10
minimal	3	3	7	3	3	0	2	5	4	3
mild	0	0	0	6	6	0	0	0	6	7
Transitional epithelium, hyperplasia										
Nasal Level I[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence	0	0	0	2	1	0	4	5	5	0
minimal	0	0	0	0	1	0	4	1	5	0
mild	0	0	0	2	0	0	0	4	0	0
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence	0	9	10	10	9	0	8	10	10	10
minimal	0	3	0	0	0	0	5	0	1	1
mild	0	6	10	10	9	0	3	10	9	9

[a] = Number of tissues examined from each group.

Text Table 10. Incidences of Changes in Respiratory Epithelium, Study Day 5 Primary Necropsy

	Males					Females				
Target Exposure Level (ppm):	**0**	**50**	**150**	**300**	**600**	**0**	**50**	**150**	**300**	**600**
Respiratory epithelium, degeneration										
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)	3 (30)
minimal	0	0	0	0	0	0	0	0	0	3
mild	0	0	0	0	1	0	0	0	0	0
Nasal Level III[a]	**10**	**10**	**10**	**10**	**10**	**10**	**8**	**10**	**9**	**10**
total incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)	2 (20)	0 (0)	0 (0)	0 (0)	0 (0)	7 (70)
minimal	0	0	0	0	2	0	0	0	0	6
mild	0	0	0	0	0	0	0	0	0	1
Nasal Level IV[a]	**8**	**7**	**6**	**6**	**9**	**6**	**7**	**4**	**7**	**4**
total incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)	1 (11)	0 (0)	0 (0)	0 (0)	0 (0)	3 (75)
minimal	0	0	0	0	1	0	0	0	0	3
Nasal Level V[a]	**9**	**8**	**8**	**10**	**10**	**10**	**9**	**10**	**9**	**10**
total incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (20)
minimal	0	0	0	0	0	0	0	0	0	1
mild	0	0	0	0	0	0	0	0	0	1
Respiratory epithelium, hyperplasia										
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence (%)	0 (0)	1 (10)	1 (10)	5 (50)	8 (80)	0 (0)	0 (0)	0 (0)	4 (40)	6 (60)
minimal	0	1	1	4	5	0	0	0	4	6
mild	0	0	0	1	3	0	0	0	0	0
Nasal Level III[a]	**10**	**10**	**10**	**10**	**10**	**10**	**8**	**10**	**9**	**10**
total incidence (%)	0 (0)	2 (20)	8 (80)	10 (100)	9 (90)	0 (0)	3 (38)	6 (60)	9 (100)	10 (100)
minimal	0	2	7	5	0	0	3	5	6	4
mild	0	0	1	5	9	0	0	1	3	6
Nasal Level IV[a]	**8**	**7**	**6**	**6**	**9**	**6**	**7**	**4**	**7**	**4**
total incidence (%)	0 (0)	0 (0)	0 (0)	5 (83)	8 (89)	0 (0)	0 (0)	2 (50)	6 (86)	4 (100)
minimal	0	0	0	5	0	0	0	2	4	1
mild	0	0	0	0	8	0	0	0	2	3
Nasal Level V[a]	**9**	**8**	**8**	**10**	**10**	**10**	**9**	**10**	**9**	**10**
total incidence (%)	0 (0)	0 (0)	0 (0)	10 (100)	10 (100)	0 (0)	0 (0)	0 (0)	6 (67)	10 (100)
minimal	0	0	0	9	1	0	0	0	5	5
mild	0	0	0	1	9	0	0	0	1	5
Nasal Level VI[a]	**10**	**9**	**10**	**9**	**8**	**10**	**10**	**10**	**8**	**10**
total incidence (%)	0 (0)	0 (0)	0 (0)	1 (11)	8 (100)	0 (0)	0 (0)	0 (0)	2 (25)	9 (90)
minimal	0	0	0	1	1	0	0	0	2	6

Mild	0	0	0	0	7	0	0	0	0	3

[a] = Number of tissues examined from each group.

Text Table 11. Incidences of Acute Inflammation in Respiratory Epithelium, Study Day 5 Primary Necropsy

	Males					**Females**				
Target Exposure Level (ppm):	**0**	**50**	**150**	**300**	**600**	**0**	**50**	**150**	**300**	**600**
Respiratory epithelium, acute inflammation										
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence (%)	2 (20)	5 (50)	4 (40)	3 (30)	5 (50)	1 (10)	0 (0)	0 (0)	3 (30)	6 (60)
minimal	2	5	4	3	5	1	0	0	3	5
mild	0	0	0	0	0	0	0	0	0	1
Nasal Level III[a]	**10**	**10**	**10**	**10**	**10**	**10**	**8**	**10**	**9**	**10**
total incidence (%)	1 (10)	0 (0)	1 (10)	5 (50)	9 (90)	0 (0)	0 (0)	1 (10)	5 (56)	8 (80)
minimal	1	0	1	5	6	0	0	1	4	5
mild	0	0	0	0	3	0	0	0	1	3
Nasal Level IV[a]	**8**	**7**	**6**	**6**	**9**	**6**	**7**	**4**	**7**	**4**
total incidence (%)	1 (13)	0 (0)	0 (0)	1 (17)	7 (78)	0 (0)	0 (0)	0 (0)	1 (14)	3 (75)
minimal	1	0	0	1	5	0	0	0	1	2
mild	0	0	0	0	2	0	0	0	0	1
Nasal Level V[a]	**9**	**8**	**8**	**10**	**10**	**10**	**9**	**10**	**9**	**10**
total incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)	2 (20)	0 (0)	0 (0)	0 (0)	0 (0)	3 (30)
minimal	0	0	0	0	2	0	0	0	0	3
Nasal Level VI[a]	**10**	**9**	**10**	**9**	**8**	**10**	**10**	**10**	**8**	**10**
total incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)	1 (13)	0 (0)	0 (0)	0 (0)	0 (0)	2 (20)
minimal	0	0	0	0	1	0	0	0	0	2

[a] = Number of tissues examined from each group.

Text Table 12. Incidences of Degeneration of Olfactory Epithelium, Study Day 5 Primary Necropsy

	Males					Females				
Target Exposure Level (ppm):	**0**	**50**	**150**	**300**	**600**	**0**	**50**	**150**	**300**	**600**
Olfactory epithelium, degeneration										
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence	0	9	10	9	8	0	9	10	10	10
minimal	0	5	1	0	1	0	6	0	0	0
mild	0	4	2	1	0	0	3	4	2	1
moderate	0	0	7	8	7	0	0	6	8	9
Nasal Level III[a]	**10**	**10**	**10**	**10**	**10**	**10**	**8**	**10**	**9**	**10**
total incidence	0	10	10	10	9	0	8	10	9	10
minimal	0	2	0	0	0	0	5	0	0	0
mild	0	8	0	0	0	0	1	2	0	0
moderate	0	0	10	10	9	0	2	8	9	10
Nasal Level IV[a]	**8**	**7**	**6**	**6**	**9**	**6**	**7**	**4**	**7**	**4**
total incidence	1	7	6	6	8	0	4	4	7	4
minimal	1	4	0	0	0	0	2	0	0	0
mild	0	3	5	1	0	0	2	2	2	0
moderate	0	0	1	5	8	0	0	2	5	4
Nasal Level V[a]	**9**	**8**	**8**	**10**	**10**	**10**	**9**	**10**	**9**	**10**
total incidence	0	6	8	10	10	0	3	10	9	10
minimal	0	6	5	1	2	0	3	9	2	0
mild	0	0	3	6	5	0	0	1	5	5
moderate	0	0	0	3	3	0	0	0	2	5
Nasal Level VI[a]	**10**	**9**	**10**	**9**	**8**	**10**	**10**	**10**	**8**	**10**
total incidence	0	2	6	8	7	0	2	4	6	8
minimal	0	2	2	4	3	0	2	4	4	6
mild	0	0	3	4	4	0	0	0	1	2
moderate	0	0	1	0	0	0	0	0	1	0

[a] = Number of tissues examined from each group.

Text Table 13. Incidences of Regeneration of Olfactory Epithelium, Study Day 5 Primary Necropsy

	Males					**Females**				
Target Exposure Level (ppm):	**0**	**50**	**150**	**300**	**600**	**0**	**50**	**150**	**300**	**600**
Olfactory epithelium, regeneration										
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence (%)	0 (0)	8 (80)	10 (100)	9 (90)	8 (80)	0 (0)	8 (80)	10 (100)	10 (100)	10 (100)
minimal	0	5	3	1	3	0	6	3	1	2
mild	0	3	7	8	5	0	2	7	9	8
Nasal Level III[a]	**10**	**10**	**10**	**10**	**10**	**10**	**8**	**10**	**9**	**10**
total incidence (%)	0 (0)	9 (90)	10 (100)	10 (100)	9 (90)	0 (0)	4 (50)	10 (100)	9 (100)	10 (100)
minimal	0	4	0	0	0	0	1	0	0	3
mild	0	5	10	10	9	0	3	10	9	7
Nasal Level IV[a]	**8**	**7**	**6**	**6**	**9**	**6**	**7**	**4**	**7**	**4**
total incidence (%)	0 (0)	4 (57)	6 (100)	6 (100)	8 (89)	0 (0)	3 (43)	4 (100)	7 (100)	4 (100)
minimal	0	3	2	0	1	0	2	0	3	0
mild	0	1	4	6	7	0	1	4	4	4
Nasal Level V[a]	**9**	**8**	**8**	**10**	**10**	**10**	**9**	**10**	**9**	**10**
total incidence (%)	0 (0)	2 (25)	8 (100)	10 (100)	10 (100)	0 (0)	2 (22)	10 (100)	9 (100)	10 (100)
minimal	0	2	8	6	7	0	2	10	7	2
mild	0	0	0	4	3	0	0	0	2	8
Nasal Level VI[a]	**10**	**9**	**10**	**9**	**8**	**10**	**10**	**10**	**8**	**10**
total incidence (%)	0 (0)	2 (22)	4 (40)	7 (78)	6 (75)	0 (0)	1 (10)	1 (10)	4 (50)	7 (70)
minimal	0	2	4	6	6	0	1	1	4	7
mild	0	0	0	1	0	0	0	0	0	0

[a] = Number of tissues examined from each group.

Text Table 14. Incidences of Acute Inflammation in Olfactory Epithelium, Study Day 5 Primary Necropsy

	Males					Females				
Target Exposure Level (ppm):	**0**	**50**	**150**	**300**	**600**	**0**	**50**	**150**	**300**	**600**
Olfactory epithelium, acute inflammation										
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence (%)	0 (0)	0 (0)	2 (20)	2 (20)	6 (60)	0 (0)	0 (0)	1 (10)	6 (60)	3 (30)
minimal	0	0	2	2	5	0	0	1	6	3
mild	0	0	0	0	1	0	0	0	0	0
Nasal Level III[a]	**10**	**10**	**10**	**10**	**10**	**10**	**8**	**10**	**9**	**10**
total incidence (%)	0 (0)	0 (0)	7 (70)	9 (90)	6 (60)	0 (0)	0 (0)	4 (40)	7 (78)	9 (90)
minimal	0	0	7	8	4	0	0	4	7	9
mild	0	0	0	1	2	0	0	0	0	0
Nasal Level IV[a]	**8**	**7**	**6**	**6**	**9**	**6**	**7**	**4**	**7**	**4**
total incidence (%)	0 (0)	1 (14)	1 (17)	2 (33)	8 (89)	0 (0)	0 (0)	1 (25)	2 (29)	3 (75)
minimal	0	1	1	2	5	0	0	1	2	1
mild	0	0	0	0	3	0	0	0	0	2
Nasal Level V[a]	**9**	**8**	**8**	**10**	**10**	**10**	**9**	**10**	**9**	**10**
total incidence (%)	0 (0)	0 (0)	0 (0)	6 (60)	4 (40)	0 (0)	0 (0)	1 (10)	4 (44)	7 (70)
minimal	0	0	0	6	3	0	0	1	4	5
mild	0	0	0	0	1	0	0	0	0	2
Nasal Level VI[a]	**10**	**9**	**10**	**9**	**8**	**10**	**10**	**10**	**8**	**10**
total incidence (%)	0 (0)	0 (0)	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (10)
minimal	0	0	1	0	0	0	0	0	0	1

[a] = Number of tissues examined from each group.

Text Table 15. Incidences of Fibro-Osseous Proliferation, Study Day 5 Primary Necropsy

	Males					Females				
Target Exposure Level (ppm):	**0**	**50**	**150**	**300**	**600**	**0**	**50**	**150**	**300**	**600**
Fibro-osseous proliferation										
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
total incidence	0	0	3	7	9	0	0	0	10	10
minimal	0	0	3	5	4	0	0	0	8	4
mild	0	0	0	2	5	0	0	0	2	6
Nasal Level III[a]	**10**	**10**	**10**	**10**	**10**	**10**	**8**	**10**	**9**	**10**
total incidence	0	0	0	0	2	0	0	0	0	2
minimal	0	0	0	0	2	0	0	0	0	2
Nasal Level IV[a]	**8**	**7**	**6**	**6**	**9**	**6**	**7**	**4**	**7**	**4**
total incidence	0	0	0	0	1	0	0	0	1	0
minimal	0	0	0	0	1	0	0	0	1	0

[a] = Number of tissues examined from each group.

Applicant's summary and conclusion

Executive summary

The objective of this study was to evaluate the potential toxic effects of dimethyl disulfide (DMDS) on tissues of the upper respiratory tract (URT) when administered as a vapor via whole-body inhalation to rats for 1 day or 5 consecutive days.

Toxicity of dimethyl disulfide (DMDS) vapors administered via 6-hour whole-body inhalation to Crl:CD(SD) rats for 1 or 5 days was observed at exposure levels of 50, 150, 300 and 600 ppm as evidenced by body weight deficits, histopathologic changes in the nasal epithelia (acute inflammation, degeneration and/or hyperplasia) and turbinate bones (fibro-osseous proliferation at 150 ppm and higher, probably secondary to inflammation), and organ weight changes in the lung (300 and 600 ppm females at the primary necropsy only). Regeneration of the olfactory epithelium despite continued exposure indicated possible recovery after cessation of exposure. The no-observed-adverse-effect level (NOAEL) for dimethyl disulfide (DMDS) vapors administered via whole-body inhalation to Crl:CD(SD) rats for 1 or 5 consecutive days was less than 50 ppm. A BMD10% of 19 ppm (with a lower limit at 95% of 9.3 ppm) was calculated for the nasal irritation.

[Study 3] Kirkpatrick (WIL) 2009/K1 KS/24-h inhalation toxicity

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	Key study	1 (reliable with restrictions)	Kirkpatrick DT	2009	A single exposure inhalation toxicity study of dimethyl disulfide (DMDS) in albino rats with microscopic examination of the upper respiratory tract

Materials and methods

Test type : other: Single 24-hour inhalation toxicity study

Limit test : no

Test guideline

Qualifier	**Guideline**	**Deviations**

no guideline followed		

Principles of method if other than guideline

Evaluation of the potential toxic effects of dimethyl disulphide (DMDS) on tissues of the upper respiratory tract (URT) when administered 24 hours as a vapor via whole-body inhalation to rats.

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.5%

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : male

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Laboratories, Inc., Raleigh, NC, USA
- Age at study initiation: 9 weeks old
- Weight at study initiation: 277 g to 346 g
- Housing: individually in clean, stainless-steel, wire-mesh cages suspended above cage-board
- Diet (ad libitum): PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002
- Water (ad libitum): Reverse osmosis treated (on site) drinking water
- Acclimation period: 10 days

ENVIRONMENTAL CONDITIONS
- Temperature : 21.5°C to 21.8°C
- Humidity: 40.6% to 44.0%
- Air changes (per hr): 10
- Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration : inhalation: vapour

Type of inhalation exposure : whole body

Vehicle : other: unchanged (no vehicle)

Details on inhalation exposure

Animal exposures were conducted in 2000-L stainless-steel and glass whole-body exposure chambers. The exposure systems were operated under dynamic conditions from a HEPA- and charcoal-filtered air source. The air flow rate through the chamber was such that there were 12-15 air changes per hour. Mean chamber temperature and relative humidity were set to be 22 ± 2°C and 30-70%, respectively. These parameters were monitored continuously and recorded approximately every 30-40 minutes throughout the exposures. Oxygen content of the exposure atmospheres was measured during the method development phase of the study, while operating at the above targeted conditions, and was at least 19%. Vapors of the test substance were generated by bubbling metered nitrogen through gas washing bottles containing appropriate aliquots of the test substance. Prior to entering the exposure chambers, the vapors of the test substance were mixed with dilution air to achieve the desired exposure levels.

Analytical verification of test atmosphere concentrations

yes (Analyzed concentrations of the test substance in each exposure chamber were determined by a specific on-line gas chromatographic method and were measured approximately every 30-40 minutes throughout the exposure period.)

Duration of exposure : 24 h

Concentrations : 5, 9, 12.5, and 18 ppm (19, 34, 48 and 69 mg/m3)

No. of animals per sex per dose : 10

Control animals : yes

Details on study design

One day following the 24-hour exposure, all animals were euthanized. All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed prior to randomization and prior to the scheduled necropsy. Individual body weights were recorded during pretest and prior to the study day 0 exposures. A final, non-fasted body weight was also recorded prior to the scheduled necropsy. Complete necropsies were conducted on all animals, and selected organs were weighed (kidneys, lungs (prior to inflation with fixative) and liver) at the scheduled necropsy. Microscopic examination was performed on nasal tissues and gross lesions from all animals in all exposure groups. Nasal sectioning was performed using the method of Morgan in which 6 nasal sections were produced using specifically defined landmarks for sectioning (Mery et al., 1994). All 6 nasal levels were examined microscopically.

Statistics

All statistical tests were performed using appropriate computing devices or programs. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test substance-treated group to the control group by sex. Each mean was presented with the standard deviation (S.D.), standard error (S.E.), and the number of animals (N) used to calculate the mean. In addition, percent difference from the control group is presented for body weights and organ weights. Due to the different rounding conventions inherent in the types of software used, the means and standard deviations on the summary and individual tables may differ by ±1 in the last significant figure. Body weight, body weight change, and organ weight data were subjected to a parametric one way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test substance treated groups to the control group.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Exp. duration	Remarks
male	other: NOAEC for nasal irritation	12.5 ppm			24 h	48 mg/m3

Mortality

All animals survived to the scheduled necropsy.

Clinical signs

There were no test substance related clinical observations.

Body weight

Body weights were unaffected by test substance exposure.

Gross pathology

There were no test substance-related macroscopic observations noted in animals at the scheduled necropsy. Organ weights were unaffected by test substance exposure.

Other findings

MICROSCOPIC EXAMINATION

Test substance exposure-related degeneration of the olfactory epithelium was observed in Nasal Levels II-VI at exposure concentrations of 12.5 and 18 ppm, and in Nasal Levels III, IV, and V at an exposure concentration of 9 ppm. Degeneration of the olfactory epithelium was also observed in a single nasal level for 2 of 10 control group animals. The degeneration in the affected nasal sections in the 9 ppm group animals consisted of single or very few discrete, extremely small foci that consisted of cellular vacuolation and individual cell pyknosis, without sloughing. Changes in

the 12.5 ppm group were also discrete areas comprising a very small percentage of the olfactory epithelium. These changes would be completely reversible and without clinical consequences and were not considered adverse. Degeneration of the olfactory epithelium was observed in 3 or more nasal levels in all 10 animals and was considered adverse in the 18 ppm group. This conclusion was based on the extent and severity of degeneration including the amount of damaged epithelium with sloughing of sensory and sustentacular cells. The lesions in this group were considered reversible. Slightly higher incidences of inflammation in the olfactory and respiratory epithelium were noted at a concentration of 18 ppm in Nasal Levels III-V. There were no test substance-related degenerative changes in the respiratory, transitional or squamous epithelium.

Any other information on results incl. tables

Results of Exposure Concentration Analyses

Parameter	Mean Concentration, ppm (% of Target) Group 2 (5 ppm)	Group 3 (9 ppm)	Group 4 (12.5 ppm)	Group 5 (18 ppm)
Nominal Concentrations	5.4 (108)	9.7 (108)	13.0 (104)	18.3 (102)
Analyzed Exposure Concentrations	5.0 (100)	8.9 (99)	12.6 (101)	18.4 (102)

Incidences of Degeneration of Olfactory Epithelium,Scheduled Necropsy (Study Day 2)

	Males				
Target Exposure Level (ppm):	**0**	**5**	**9**	**12.5**	**18**
Olfactory epithelium, degeneration					
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**
Total incidence	0	0	0	2	5
minimal	-	-	-	2	2
mild	-	-	-	0	2
moderate	-	-	-	0	1
Nasal Level III[a]	**10**	**10**	**10**	**10**	**10**
Total incidence	1	0	2	2	10
minimal	1	-	2	2	5
mild	0	-	0	0	2
moderate	0	-	0	0	3
Nasal Level IV[a]	**10**	**10**	**10**	**10**	**10**
Total incidence	1	0	2	5	10
minimal	1	-	2	5	3
mild	0	-	0	0	7
Nasal Level V[a]	**10**	**10**	**10**	**10**	**10**
Total incidence	0	0	2	6	10
minimal	-	-	2	6	9
mild	-	-	0	0	1
Nasal Level VI[a]	**10**	**10**	**10**	**10**	**10**
Total incidence	0	0	0	3	4
minimal	-	-	-	3	3
mild	-	-	-	0	1

[a]= Number of tissues examined from each group

Number of Nasal Levels with Degeneration of Olfactory EpitheliumScheduled Necropsy (Study Day 2)

	Males				
Target Exposure Level (ppm):	**0**	**5**	**9**	**12.5**	**18**
Olfactory epithelium, degeneration[a]	**2**	**0**	**4**	**6**	**10**
One level	2	0	3	1	0
Two levels	0	0	0	1	0
Three levels	0	0	1	2	2
Four levels	0	0	0	1	7
Five levels	0	0	0	1	1

[a]= Number of animals with olfactory epithelial degeneration in at least 1 nasal level

Incidences of Inflammation of Olfactory andRespiratory Epithelia in Selected Nasal

LevelsScheduled Necropsy (Study Day 2)					
	Males				
Target Exposure Level (ppm):	**0**	**5**	**9**	**12.5**	**18**
Nasal Level II[a]	**10**	**10**	**10**	**10**	**10**
Olfactory epithelium, inflammation, minimal	0	0	0	0	1
Respiratory epithelium, inflammation, minimal	1	0	0	0	0
Nasal Level III[a]	**10**	**10**	**10**	**10**	**10**
Olfactory epithelium, inflammation, minimal	0	0	0	2	4
Respiratory epithelium, inflammation, minimal	1	1	1	1	4
Nasal Level IV[a]	**10**	**10**	**10**	**10**	**10**
Olfactory epithelium, inflammation, minimal	2	0	3	3	5
Respiratory epithelium, inflammation, minimal	0	1	1	2	3
Nasal Level V[a]	**10**	**10**	**10**	**10**	**10**
Olfactory epithelium, inflammation, minimal	1	0	0	0	3
Respiratory epithelium, inflammation, minimal	1	1	0	0	6
Nasal Level VI[a]	**10**	**10**	**10**	**10**	**10**
Olfactory epithelium, inflammation, minimal	0	0	1	0	1
Respiratory epithelium, inflammation, minimal	0	0	1	0	0

[a]= Number of tissues examined from each group

Applicant's summary and conclusion

Conclusions

Dimethyl disulphide (DMDS) vapors administered via a 24-hour whole-body inhalation to Crl:CD(SD) rats resulted in test substance exposure related degeneration of the olfactory epithelium at exposure levels of 9, 12.5, and 18 ppm and a slight increase in inflammation of the respiratory and olfactory epithelia at 18 ppm. All findings of olfactory epithelial degeneration in the 9 and 12.5 ppm group animals were graded as minimal and considered non-adverse based on the very limited extent and severity of the lesions and the finding of degeneration in 2 control animals. Although all of the changes in the test substance-exposed groups were considered reversible, degeneration of the olfactory epithelium was considered adverse for the 18 ppm group based on the extent and severity of the lesions. Therefore, the no observed adverse-effect concentration (NOAEC) for 24-hour whole-body exposure of DMDS to Crl:CD(SD) rats was 12.5 ppm.

Executive summary

Dimethyl disulphide (DMDS) was administered as a 24-hour, whole-body exposure to 4 groups (Groups 2-5) of Crl:CD(SD) rats. Target exposure concentrations were 5, 9, 12.5, and 18 ppm (19, 34, 48 and 69 mg/m3) for Groups 2, 3, 4, and 5, respectively. A concurrent control group (Group 1) was exposed to filtered air on a comparable regimen. The control group (Group 1) and Groups 2-5 each consisted of 10 males. One day following the 24-hour exposure, all animals were euthanized. All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed prior to randomization and prior to the scheduled necropsy. Individual body weights were recorded during pretest and prior to the study day 0 exposures. A final, non-fasted body weight was also recorded prior to the scheduled necropsy. Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsy. Selected tissues were examined microscopically from all animals. All animals survived to the scheduled necropsy. No clinical or macroscopic findings attributed to test substance exposure were noted at any exposure concentration. Body weight was unaffected by test substance exposure. Test substance exposure-related degeneration of the olfactory epithelium was observed in Nasal Levels II-VI at exposure concentrations of 12.5 and 18 ppm, and in Nasal Levels III, IV, and V at an exposure concentration of 9 ppm. Degeneration of the olfactory epithelium was also observed in a single nasal level for 2 of 10 control group animals. The degeneration in the affected nasal sections in the 9 ppm group animals consisted of single or very few discrete, extremely small foci that consisted of cellular vacuolation and individual cell pyknosis, without sloughing. Changes in the 12.5 ppm group were also discrete areas comprising a very small percentage of the olfactory epithelium. These changes would be completely reversible and without clinical consequences and were not considered adverse. Degeneration of the olfactory epithelium was observed in 3 or more nasal levels in all 10 animals and was considered adverse in the 18 ppm group. This conclusion was based on the extent and severity of degeneration including the amount of damaged epithelium with sloughing of sensory and sustentacular cells. The lesions in this group were considered reversible. Slightly higher incidences of inflammation in the olfactory and respiratory epithelium were noted at a concentration of 18 ppm in Nasal Levels III-V. There were no test substance-related degenerative changes in the respiratory, transitional or squamous epithelium. The no-observed-adverse-effect concentration

(NOAEC) for 24-hour whole-body exposure of DMDS to Crl:CD(SD) rats was 12.5 ppm (48 mg/m3).

3.4 Acute toxicity – Other routes

3.5 Skin corrosion/irritation

[Study 1] Guillot (Hazleton-IFT) 1985/K1 KS /Skin irritation, corrosion

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable with restrictions)	Guillot JP	1985	DISULFURE DE DIMETHYLE. Tests de tolérance locale chez le lapin, Irritation primaire cutanée

Materials and methods

Type of method : in vivo

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	no

GLP compliance : no

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 98.98%

Test animals

Species : rabbit

Strain : New Zealand White

Details on test animals and environmental conditions
TEST ANIMALS
- Source: no data
- Age at study initiation: no data
- Weight at study initiation: 2.5 +/- 0.2 kg
- Housing: individually in polystyrene cage (540x360x315xmm)
- Diet : granulé Lapin entretien "112", UAR, ad libitum
- Water: ad libitum
- Acclimation period: 8 days
ENVIRONMENTAL CONDITIONS
- Temperature (°C): no data
- Humidity (%): no data
- Air changes (per hr): no data
- Photoperiod (hrs dark / hrs light): no data
IN-LIFE DATES: 1985-02-26 To: 1985-02-29

Test system

Type of coverage : semiocclusive

Preparation of test site : shaved

Vehicle : unchanged (no vehicle)

Amount/concentration applied : 0.5 ml

Duration of treatment / exposure : 4 hour(s)

Observation period : 72 hours

Number of animals : 6

Control animals : not required

Details on study design

0.5 ml of the test substance, applied with a sterile polypropylene syringe of 2 ml, on a Codex hydrophilic eight layer gauze pad, about 2.5 cm square, previously laid down on the skin to avoid any loss of test substance. The gauze pad was held in contact with the skin with a semi-occlusive patch.

Results and discussions

Irritation / corrosion results

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
erythema score	mean	24+48+72 h	1.78	4	not fully reversible within: 72 h	
edema score	mean	24+48+72 h	1.22	4	not fully reversible within: 72h	

Individual and mean skin irritation scores

Animal No	Erythema/Oedema 11000	11001	11002	11005	11010	11042
after 1 h	2 / 1	1/ 2	1 / 2	1 / 1	1 /1	2 / 2
after 24 h	2 / 2	2* / 1	2* / 2	2* / 2	2 / 2	2* / 2
after 48 h	2 / 1	2* / 1	2* / 2	1* / 1	2 / 1	2* / 2
after 72 h	1 / 0	2* / 1	2* / 1	1* / 0	1 / 0	2* / 1
mean score 24/48/72 h	1.67 / 1	2 / 1	2 / 1.67	1.33 / 1	1.67 / 1	2 / 1.67

*= reaction is seen beyond the area of application

Applicant's summary and conclusion

Interpretation of results : slightly irritating (not classified)

Criteria used for interpretation of results : other: REGULATION (EC) No 1272-2008

Conclusions

Dimethyl disulphide was slightly irritating. The effects were not fully reversible until the end of the observation period (72 hours). However, another study (Shapiro, 1985) indicated an full reversibility within 10 days. Accordingly, dimethyl disulphide don't need to be classified for skin irritation.

Executive summary

The acute dermal irritation of neat dimethyl disulphide was evaluated in rabbits according to OECD 404 guideline. Dimethyl disulphide was applied undiluted to the skin of 6 New-Zealand White albino rabbits and held in contact for 4 hours by means of a semi-occlusive dressing. Mean scores over 24, 48 and 72 hours were 1.78 and 1.22 for erythema and for edema, respectively. Under these experimental conditions, dimethyl disulphide was slightly irritating when applied topically to rabbits.

[Study 2] Shapiro (PSL) 1985/K4 WoE/Skin irritation, corrosion

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report		4 (not assignable)	Shapiro R	1985	DIMETHYL DISULFIDE, EPA primary skin irritation

Materials and methods

Type of method : in vivo

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: EPA 40 CFR 163.81-5	no

GLP compliance : yes

Test materials

DMDS tested in this study did not correspond to the specifications of DMDS proposed for registration (high level of methyl mercaptan).

Test animals

Species : rabbit

Strain : New Zealand White

Details on test animals and environmental conditions

TEST ORGANISMS: - Source: Davidson's Mill Farm, S. Brunswick, NJ - Age: no data - Weight at study initiation: 3.2-3.5 kg - Adaptation period: 8 weeks - Number of animals: 4 males + 2 females HOUSING The animals were housed individually in wire bottomed cages FOOD and WATER - Food: Purina rabbit pellets, ad libitum - Water: ad libitum ENVIRONMENTAL CONDITIONS - Temperature : 68-72°F - Relative humidity : no data - Light/dark cycle : 12h/12h - Ventilation : no data

Test system

Type of coverage : occlusive

Preparation of test site : shaved

Vehicle : unchanged (no vehicle)

Amount/concentration applied : 0.5 ml

Duration of treatment / exposure : 24

hour(s)

Observation period : 14 days

Number of animals : 6

Control animals : not required

Details on study design

Six healthy rabbits were prepared by clipping the back and trunk (from the scapular to the lumbar region), free of hair. Two 2.5 cm2 gauze patches were placed over intact skin on each rabbit. Five-tenths of a milliliter of the test material (neat) was placed under each patch. All the patches were secured in place with adhesive tape and wrapped with an occlusive elastic sleeve. The rabbits were then placed in Elizabethan collars for 24 hours to prevent them from disturbing the wrapping and test sites. Following 24 hours of exposure, the wrappings and gauze squares were removed and the test site was gently wiped free of excess test material. At approximately 1, 24, 48 and 72 hours, and 7, 10 and 14 days after removal of the patches, the skin was evaluated for dermal irritation (erythema, edema or other evidence of skin irritation) according to the Draize scoring system. Special care was taken to note any signs of necrosis, eschar or other evidence of irreversible alteration of tissue structure. Observations for any gross signs of abnormal pharmacologic or toxic effects were also made.

Results and discussions

Irritation / corrosion results

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
erythema score	mean	24+48+72 h	1.03	4	fully reversible within: 10 days	
edema score	mean	24+48+72 h	0.11	4	fully reversible within: 48 h	

Irritant/corrosive response data

Slight to moderate erythema was observed on the intact sites at 24 hours. Slight edema was observed in five rabbits. The same conditions persisted at 48 and 72 hours except that edema disappeared within 48 hours after patch application. By 7 days erythema decreased greatly or completely disappeared. All erythema disappeared by day 10. By day 14, all rabbits appeared normal with no evidence of erythema or edema.

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Page No. 4

Report No. T-5149

	Exposure Time	Average Exposure Units	Exposure Time	Average Exposure Units	Exposure Time	Average Exposure Units
	hours	value	days	value	days	value
Erythema and Eschar Formation						
Abraded Skin	24	2.50	72 hrs.	2.17	10	0.33
Do	48	2.25	7	1.42	14	0.00
Intact Skin	24	1.17	72 hrs.	0.92	10	0.00
Do	48	1.00	7	0.33	14	0.00
Subtotal		6.92		4.84		0.33
Edema Formation						
Abraded Skin	24	0.83	72 hrs.	0.00	10	0.00
Do	48	0.00	7	0.00	14	0.00
Intact Skin	24	0.33	72 hrs.	0.00	10	0.00
Do	48	0.00	7	0.00	14	0.00
Subtotal		1.16		0.00		0.00
Total		8.08		4.84		0.33
Primary Irritation Score		2.02		1.21		0.08
Average Primary Irritation Score (14 days)		1.10				

Primary Irritation Score: (Skin)

0.5	- non-primary irritant
0.5 - 2.0	- mild primary irritant
2.1 - 5.0	- moderate primary irritant
5.1	- severe primary irritant

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Page No. 5
Report No. T-5149

INDIVIDUAL RESULTS

Erythema and Eschar Formation

Rabbit No.	Patch No.	24 hours Abraded	24 hours Intact	~~72 hours~~ 48hr. Abraded	~~72 hours~~ 48hr. Intact	~~4 days~~ 72hr. Abraded	~~4 days~~ 72hr. Intact
3814	1	4	0	4	0	4	0
	2	4	2	4	2	4	2
3815	1	2	2	2	2	2	1
	2	3	2	3	2	2	2
3816	1	2	1	2	1	2	1
	2	2	1	2	1	2	1
3817	1	1	1	1	1	1	1
	2	2	1	2	1	2	1
3818	1	4	1	2	1	3	1
	2	2	1	3	1	2	1
3819	1	2	1	1	0	1	0
	2	2	1	1	0	1	0
Total		30	14	27	12	26	11
Patch Average		2.50	1.17	2.25	1.00	2.17	0.92

Rabbit No.	Patch No.	7 days Abraded	7 days Intact	10 days Abraded	10 days Intact	14 days Abraded	14 days Intact
3814	1	4	0	1	0	0	0
	2	4	2	1	0	0	0
3815	1	0	0	0	0	0	0
	2	1	0	0	0	0	0
3816	1	1	0	0	0	0	0
	2	1	0	0	0	0	0
3817	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
3818	1	2	1	1	0	0	0
	2	2	1	1	0	0	0
3819	1	1	0	0	0	0	0
	2	1	0	0	0	0	0
Total		17	4	4	0	0	0
Patch Average		1.42	0.33	0.33	0.0	0.0	0.0

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Page No. 6
Report No. T-5149

Edema Formation

Rabbit No.	Patch No.	24 hours Abraded	24 hours Intact	~~72~~ 48 hr hours Abraded	~~72~~ 48 hr hours Intact	~~4~~ 72 hr days Abraded	~~4~~ 72 hr days Intact
3814	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
3815	1	1	0	0	0	0	0
	2	2	2	0	0	0	0
3816	1	2	0	0	0	0	0
	2	1	0	0	0	0	0
3817	1	2	1	0	0	0	0
	2	0	0	0	0	0	0
3818	1	1	1	0	0	0	0
	2	0	0	0	0	0	0
3819	1	1	0	0	0	0	0
	2	0	0	0	0	0	0
Total		10	4	0	0	0	0
Patch Average		0.83	0.33	0.0	0.0	0.0	0.0

Rabbit No.	Patch No.	7 days Abraded	7 days Intact	10 days Abraded	10 days Intact	14 days Abraded	14 days Intact
3814	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
3815	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
3816	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
3817	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
3818	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
3819	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
Total		0	0	0	0	0	0
Patch Average		0.0	0.0	0.0	0.0	0.0	0.0

Applicant's summary and conclusion

Interpretation of results : slightly irritating (Not classified)

Criteria used for interpretation of results : other: REGULATION (EC) No 1272-2008

Conclusions

Dimethyl Disulfide is considered to be slightly irritating.

Executive summary

The acute dermal irritation of dimethyl disulphide (DMDS) was evaluated in rabbits according to EPA 40 CFR 163.81-5 guideline. DMDS was applied undiluted to the intact skin of 6 New-Zealand White albino rabbits and held in contact for 24 hours by means of an occlusive dressing. The mean scores over 24, 48 and 72 hours were 1.03 and 0.11 for erythema and for edema, respectively. All lesions were reversible within 10 days.Under these experimental conditions, DMDS was slightly irritating when applied topically to rabbits.

3.6 Serious eye damage/eye irritation

[Study 1] Guillot (Hazleton-IFT) 1985/K1 KS/Eye irritation

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key Stduy	1 (reliable without restriction)	Guillot JP	1985	DISULFURE DE DIMETHYLE. Tests de tolérance locale chez le lapin, irritation oculaire

Materials and methods

Type of method :
in vivo

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 405 (Acute Eye Irritation / Corrosion)	no

GLP compliance : no

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 98.98%

Test animals

Species : rabbit

Strain : New Zealand White

Details on test animals and environmental conditions

TEST ANIMALS
- Source: no data
- Age at study initiation: no data
- Weight at study initiation: 2.5 +/- 0.2 kg
- Housing: individually in polystyrene cage (540x360x315xmm)

- Diet : granulé Lapin entretien "112", UAR, ad libitum
- Water: ad libitum
- Acclimation period: 8 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): no data
- Humidity (%): no data
- Air changes (per hr): no data
- Photoperiod (hrs dark / hrs light): no data

IN-LIFE DATES: 1985-03-07 To: 1985-03-10

Test system

Vehicle : unchanged (no vehicle)

Amount/concentration applied : 0.1 ml

Duration of treatment / exposure : 24 hour(s)

Observation period :72 hours

Number of animals : 6

Control animals : not required

Details on study design :Comment: not rinsed

Results and discussions

Overall irritation / corrosion results

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
conjunctivae score (enanthema)	mean	24+48+72 h	1.33	3	not fully reversible within: 72 h	
chemosis score	mean	24+48+72 h	1.89	4	not fully reversible within: 72 h	
iris score	mean	24+48+72 h	1	2	not fully reversible within: 72 h	
cornea score	mean	24+48+72 h	0.83	4	not fully reversible within: 72 h	

TEST D'IRRITATION OCULAIRE CHEZ LE LAPIN - RÉSULTATS

(OCULAR IRRITATION TEST IN THE RABBIT - RESULTS)

PRODUIT *(Test substance)* : DISULFURE DE DIMETHYLE (DMDS) - LOT A1 -

APPLICATION : 0,1 ml par animal du produit tel quel, sans rinçage

DATE DE L'INSTILLATION *(Date of instillation)* : 7 MARS 1985 - 12 h 45

LECTURES *(Readings)*	LAPINS N° *(Rabbits N°)*	CONJONCTIVE *(Conjunctiva)*		IRIS		CORNÉE *(Cornea)*		
		Chemosis *(Chaemosis)*	Enanthème *(Enanthema)*	Réflexe *(Reflex)*	Congestion *(Congestion)*	Opacité *(Opacity)* Degre *(Degree)*	Opacité *(Opacity)* Surface *(Area)*	Ulcération *(Ulceration)*
1 H	11208	2	2	N*	1p	0	0	0
	11211	2	1	N*	1p	0	0	0
	11216	2	1	N	1c	0	0	0
	11217	2	1	N	1p	2	1	0
	11223	2	2	N	1p	2	1	0
	11230	2	2	N	1p	2	1	0
24 H	11208	2	2	N	1p	2	1	0
	11211	2	2	N*	1p	2	1	0
	11216	2	1	N	1p	0	0	0
	11217	2	1	N	1c	2	1	0
	11223	2	2	N*	1p	2	2	U
	11230	2	1	N*	1p	2	1	0
	Moyennes *(Means)*	2,00	1,50		1,00	1,67		
48 H	11208	2	2	N	1c	0	0	0
	11211	2	2	N	1p	2	1	0
	11216	2	1	N	1c	0	0	0
	11217	2	1	N	1c	0	0	0
	11223	2	2	N	1p	1	1	0
	11230	2	1	N	1p	0	0	0
	Moyennes *(Means)*	2,00	1,50		1,00	0,50		
72 H	11208	2	2	N	1c	0	0	0
	11211	2	1	N	1p	1	1	0
	11216	1	0	N	1c	0	0	0
	11217	2	1	N	1c	0	0	0
	11223	1	1	N	1c	1	1	0
	11230	2	1	N	1c	0	0	0
	Moyennes *(Means)*	1,67	1,00		1,00	0,33		
MOYENNES *(Means)* 24 H + 48 H + 72 H		1,89	1,33		1,00	0,83		

OBSERVATIONS :

* : Myosis permanent avec conservation du réflexe photomoteur direct
p : Injections circumcornéales + congestion de l'iris
c : Injections circumcornéales

Applicant's summary and conclusion

Interpretation of results : Category 2 (irritating to eyes)

Criteria used for interpretation of results : REGULATION (EC) No 1272-2008

Conclusions

Dimethyl disulphide is considered as irritating for the eyes.

Executive summary

The potential of dimethyl sulphide to induce ocular irritation was evaluated in rabbits according to OECD (No. 405) guideline.A single dose of 0.1mL of the undiluted test item was instilled into the conjunctival sac of the eye of 6 rabbits. The eyes were not rinsed after administration of the test item.
Ocular reactions were observed approximately 1 hour, 24, 48 and 72 hours after the administration. Mean scores calculated over 24, 48 and 72 hours were 1.89 for chemosis, 1.33 for redness of the conjunctiva, 1.00 for iris lesions and 0.83 for corneal opacity. Under these experimental conditions, dimethyl disulphide was irritant when administered by ocular route to rabbits.

[Study 2] Shapiro (PSL) 1985/K4 WoE/Eye irritation

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	weight of evidence	4 (not assignable)	Shapiro R	1985	DIMETHYL DISULFIDE, EPA primary eye irritation.

Materials and methods

Type of method : in vivo

Test guideline

Qualifier	**Guideline**	**Deviations**
according to	other guideline: EPA-40 CFR 163-81-4	no

GLP compliance : yes

Test materials

DMDS tested in this study did not correspond to the specifications of DMDS proposed for registration (high level of methyl mercaptan).

Test animals

Species : rabbit

Strain : New Zealand White

Details on test animals and environmental conditions

TEST ORGANISMS: - Source: Davidson's Mill Farm, S. Brunswick, NJ - Age: no data - Weight at study initiation: 2.3-3.0 kg - Adaptation period: 7 days - Number of animals: 4 males + 5 females HOUSING The animals were housed individually in wire bottomed cages FOOD and WATER - Food: Purina rabbit pellets, ad libitum - Water: ad libitum ENVIRONMENTAL CONDITIONS - Temperature : 68-72°F - Relative humidity : no data - Light/dark cycle : 12h/12h - Ventilation : no data

Test system

Vehicle : unchanged (no vehicle)

Amount/concentration applied : 0.1 ml

Duration of treatment / exposure :not rinced for 6 rabbits, rinced after 20-30 sec. for 3 rabbits

Observation period : 21 days

Number of animals : 9 (6 not rinced, 3 rinced)

Control animals : not required

<u>Results and discussions</u>

Overall irritation / corrosion results

Irritation parameter	**Basis**	**Time point**	**Score**	**Max. score**	**Reversibility**	**Remarks**
conjunctivae score (Unwashed)	mean	24+48+72 h	1.32	3	fully reversible within: 7 days	
chemosis score (Unwashed)	mean	24+48+72 h	1	4	fully reversible within: 7 days	
cornea score (Unwashed)	mean	24+48+72 h	0.33	4	fully reversible within: 7 days	
iris score (Unwashed)	mean	24+48+72 h	0	2		
conjunctivae score (Washed)	mean	24+48+72 h	0.66	3	fully reversible within: 72 h	
chemosis score (Washed)	mean	24+48+72h	0.44	4	fully reversible within: 72 h	
iris score (Washed)	mean	24+48+72h	0	2		
cornea score (Washed)	mean	24+48+72h	0	4		

Irritant/corrosive response data

Corneal opacity was observed in the unwashed eyes of 2 rabbits. The opacity was transient and disappeared by 7 days. Transient hyperemia and chemosis were also observed in all rabbits (washed and un washed eyes). These conditions disappeared between 24 and 96 hours post instillation. No iridial damage was noted in any of the rabbits.

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Page No. 5
Report No. T-5148

(UNWASHED)

RESULTS: (Draize Scores)[1]

Rabbit No. 3820

Hours:	1 hr	24 hr	48 hr	72 hr	4 da	7 da	10 da	14 da	17 da	21 da
I. Cornea										
A. Opacity	0	0	0	0	0	0	0			
B. Area	4	4	4	4	4	4	4			
AxBx5	0	0	0	0	0	0	0			
II. Iris										
A. Values	0	0	0	0	0	0	0			
Ax5	0	0	0	0	0	0	0			
III. Conjunctivae										
A. Hyperemia	3	3	1	1	1	0	0			
B. Chemosis	3	4	1	0	0	0	0			
C. Discharge	2	2	0	0	0	0	0			
(A+B+C)x2	16	18	4	2	2	0	0			
Total	16	18	4	2	2	0	0			
Total Possible 110										
MTS (24 hr.)[2]		18								

Rabbit No.: 3821

Hours:	1 hr	24 hr	48 hr	72 hr	4 da	7 da	10 da	14 da	17 da	21 da
I. Cornea										
A. Opacity	0	0	0	0	0	0	0			
B. Area	4	4	4	4	4	4	4			
AxBx5	0	0	0	0	0	0	0			
II. Iris										
A. Values	0	0	0	0	0	0	0			
Ax5	0	0	0	0	0	0	0			
III. Conjunctivae										
A. Hyperemia	3	3	0	0	0	0	0			
B. Chemosis	2	3	1	1	0	0	0			
C. Discharge	2	0	0	0	0	0	0			
(A+B+C)x2	14	12	2	2	0	0	0			
Total	14	12	2	2	0	0	0			
Total Possible 110										
MTS (24 hr.)		12								

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Page No. 6
Report No. T-5148

(UNWASHED)

RESULTS: (Draize Scores)[1]

Rabbit No. 3822

Hours:	1 hr	24 hr	48 hr	72 hr	4 da	7 da	10 da	14 da	17 da	21 da
I. Cornea										
A. Opacity	0	0	0	0	0	0	0			
B. Area	4	4	4	4	4	4	4			
AxBx5	0	0	0	0	0	0	0			
II. Iris										
A. Values	0	0	0	0	0	0	0			
Ax5	0	0	0	0	0	0	0			
III. Conjunctivae										
A. Hyperemia	3	3	1	1	0	0	0			
B. Chemosis	2	2	0	0	0	0	0			
C. Discharge	2	0	0	0	0	0	0			
(A+B+C)x2	14	10	2	2	0	0	0			
Total	14	10	2	2	0	0	0			
Total Possible 110										
MTS (24 hr.)[2]		10								

Rabbit No.: 3823

Hours:	1 hr	24 hr	48 hr	72 hr	4 da	7 da	10 da	14 da	17 da	21 da
I. Cornea										
A. Opacity	0	1	0	0	0	0	0			
B. Area	4	1	4	4	4	4	4			
AxBx5	0	5	0	0	0	0	0			
II. Iris										
A. Values	0	0	0	0	0	0	0			
Ax5	0	0	0	0	0	0	0			
III. Conjunctivae										
A. Hyperemia	3	3	1	1	0	0	0			
B. Chemosis	2	1	0	0	0	0	0			
C. Discharge	2	0	0	0	0	0	0			
(A+B+C)x2	14	8	2	2	0	0	0			
Total	14	13	2	2	0	0	0			
Total Possible 110										
MTS (24 hr.)		13								

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Page No. 7
Report No. T-5148

(UNWASHED)

RESULTS: (Draize Scores)[1]

Rabbit No. 3824

Hours:	1 hr	24 hr	48 hr	72 hr	4 da	7 da	10 da	14 da	17 da	21 da
I. Cornea										
A. Opacity	0	0	0	0	0	0	0			
B. Area	4	4	4	4	4	4	4			
AxBx5	0	0	0	0	0	0	0			
II. Iris										
A. Values	0	0	0	0	0	0	0			
Ax5	0	0	0	0	0	0	0			
III. Conjunctivae										
A. Hyperemia	2	1	0	0	0	0	0			
B. Chemosis	2	1	0	0	0	0	0			
C. Discharge	2	0	0	0	0	0	0			
(A+B+C)x2	12	4	0	0	0	0	0			
Total	12	4	0	0	0	0	0			
Total Possible 110										
MTS (24 hr.)[2]		4								

Rabbit No.: 3825

Hours:	1 hr	24 hr	48 hr	72 hr	4 da	7 da	10 da	14 da	17 da	21 da
I. Cornea										
A. Opacity	0	2	2	1	1	0	0			
B. Area	4	2	1	1	1	4	4			
AxBx5	0	20	10	5	5	0	0			
II. Iris										
A. Values	0	0	0	0	0	0	0			
Ax5	0	0	0	0	0	0	0			
III. Conjunctivae										
A. Hyperemia	3	3	1	1	1	0	0			
B. Chemosis	3	2	1	1	1	0	0			
C. Discharge	2	1	0	0	0	0	0			
(A+B+C)x2	16	12	4	4	4	0	0			
Total	16	32	14	9	9	0	0			
Total Possible 110										
MTS (24 hr.)		32								

Applicant's summary and conclusion

Interpretation of results : slightly irritating

Conclusions

Dimethyl Disulphide is considered to be slightly irritating to both the unwashed and the washed eye.

Executive summary

The potential of dimethyl sulphide to induce ocular irritation was evaluated in rabbits according to EPA-40 CFR 163-81-4 guideline.A single dose of 0.1mL of the undiluted test item was instilled into the conjunctival sac of the eye of 9 rabbits. The eyes were for 6 rabbits or rinced after 20-30 sec. for 3 rabbits after administration. Ocular reactions were observed approximately 1 hour, 24, 48 and 72 hours after the administration.Corneal opacity was observed in the unwashed eyes of 2 rabbits. The opacity was transient and disappeared by 7 days. Transient hyperemia and chemosis were also observed in all rabbits (washed and un washed eyes). These conditions disappeared between 24 and 96 hours post instillation. No iridial damage was noted in any of the rabbits.
Mean scores calculated over 24, 48 and 72 hours for the unwashed eyes were 1.0 for chemosis, 1.33 for redness of the conjunctiva, 0.0 for iris lesions and 0.33 for corneal opacity. Under these experimental conditions, dimethyl disulphide was considered slightly irritating to the eyes.

3.7 Skin sensitisation

[Study 1] Rokh (CIT) 2012/K1 KS/OECD 429

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	key study	1 (reliable without restriction)	Rokh N	2012	Evaluation of skin sensitization potential in mice using the Local Lymph Node Assay (LLNA)

Materials and methods

Type of method : other: ex vivo

Type of study : Mouse local lymphnode assay (LLNA)

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay)	no
according to	EU Method B.42 (Skin Sensitisation: Local Lymph Node Assay)	no

GLP compliance : yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Purity: 99.8%

Test animals

Species : mouse

Strain : CBA

Sex : female

Details on test animals and environmental conditions
TEST ANIMALS
- Source: breeder: Janvier, Le Genest-Saint-Isle, France
- Age at study initiation: on the beginning of the treatment period, the animals of the preliminary test were approximately 10-12 weeks old and the animals of the main test were approximately 8 weeks old
- Mean body weight at study initiation: in the main test, they had a mean body weight of 20.0 g (range: 18.8 g to 21.0 g)
- Fasting period before study: no
- Housing: polycarbonate cages
- Diet: SSNIFF R/M-H pelleted diet (free access)
- Water: tap water filtered with a 0.22 µm filter (free access)
- Acclimation period: 6 days before the beginning of the study.
ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22 ± 2°C
- Humidity (%): 50 ± 20%
- Air changes (per hr): approximately 12 cycles/hour of filtered, non-recycled air
- Photoperiod (hrs dark / hrs light): 12 h/12 h (7:00 - 19:00)
IN-LIFE DATES: 15 February 2012 to 27 February 2012.

Test system

LLNA
Vehicle : acetone/olive oil (4:1 v/v)

Concentration : Range-finding test: 10%, 25%, 50% and 100%.Main test: 2.5%, 5%, 10%, 25% and 50%.

No. of animals per dose : 4 per dose.

Details on study design (LLNA)
RANGE FINDING TESTS:
- Compound solubility: In order to select the most appropriate concentration, the solubility assay first started at the concentration of 50%.A solution was obtained at the concentration of 50% in AOO. As the test item is a liquid that can be sampled using a pipette, the maximum achievable concentration was 100%.
- Irritation: Female treated at 100% was found dead on day 3. Dryness of the skin was noted on day 2 in female treated at 100%.No notable increase in ear thickness was observed at any tested concentrations.The highest concentration retained for the main test was therefore 50%.
MAIN STUDYANIMAL ASSIGNMENT AND TREATMENT
- Name of test method: murine Local Lymph Node Assay- Criteria used to consider a positive response: stimulation Index SI >= 3 and dose-relationship; additional consideration of ear thickness
TREATMENT PREPARATION AND ADMINISTRATION:
- Treatment preparation: The test item was prepared at the chosen concentrations in the vehicle. The positive control was dissolved in AOO at the concentration of 25% (v/v).
- Administration:On days 1, 2 and 3, a dose-volume of 25 µL of the control or dosage form preparations was applied to the dorsal surface of both ears, using an adjustable pipette fitted with a plastic tip.In order to avoid licking and to ensure an optimized application of the test materials, the animals were placed under light isoflurane anesthezia during the administration. No massage was performed but the tip was used to spread the preparation over the application sites. No rinsing was performed between each application.

Positive control substance(s) :hexyl cinnamic aldehyde (CAS No 101-86-0)

Results and discussion

Positive control results : The threshold positive value of 3 for the SI was reached in the positive control group (see Executive summary).

LLNA
Disintegrations per minute (DPM)

	DPM per group:
Group 5: Vehicle:	1080 / 1203
Group 6: 2.5%:	3228 / 4065
Group 7: 5%:	2594 / 2889
Group 8: 10%:	2056 / 2224
Group 9: 25%:	3566 / 4302
Group 10: 50%:	5129 / 5737

... / ...: first counting / second counting

Stimulation index : The EC3 value is approximately 2.50%.

Treatment	Concentration (%)	Irritation level	Stimulation Index (SI)
Test item	2.5	I	2.99 / 3.38
Test item	5	I	2.40 / 2.40
Test item	10	I	1.90 / 1.85
Test item	25	I	3.30 / 3.58
Test item	50	I	4.75 / 4.77
HCA	25	-	20.98 / 19.98

-: not recorded
I: non-irritant (increase in ear thickness < 10%)
…/…: first counting / second counting

Applicant's summary and conclusion

Interpretation of results : sensitising (category 1B)

Criteria used for interpretation of results : EU

Conclusions

Dimethyl disulphide induced delayed contact hypersensitivity in the murine Local Lymph Node Assay.According to the EC3 value obtained, the test item should be considered as a moderate sensitizer.

Executive summary

The objective of this study was to evaluate the potential of dimethyl disulphideto induce delayed contact hypersensitivity using the murine Local Lymph Node Assay (LLNA).This study was conducted in compliance with the principles of Good Laboratory Practice.
To assess the irritant potential of the test item (through ear thickness measurement), a preliminary test was first performed in order to define the test item concentrations to be used in the main test. Four groups of one female mouse received the test itemby topical route to the dorsal surface of both ears (one concentration per animal) on days 1, 2 and 3 at concentrations of 10, 25, 50 or 100% under a dose-volume of 25 µL.From day 1 to day 3 then on day 6, the thicknessof both ears of each animal was measured and the local reactions were recorded. Each animal was observed once a day for mortality and clinical signs. Body weight was recordedonce during the acclimation period, and thenon days 1 and 6.On completion of the observation period, surviving animals were sacrificedthen discarded without macroscopic*post-mortem*examination.
In the main test, five groups of four female mice received the test item by topical routeto the dorsal surface of both earson days 1, 2 and 3at concentrations of 2.5, 5, 10, 25 or 50% under a dose-volume of 25 µL. One negative control group of four females received the vehicle (acetone/olive oil (4/1; v/v)) under the same experimental conditions. Additionally, one positive control group of four females received the positive control, α-hexylcinnamaldehyde (HCA), at 25%in a mixture acetone/olive oil (4/1; v/v) under the same experimental conditions.
From day 1 to day 3 then on day 6, the thicknessof the left ear of each animal was measured, except in animals of the positive control group, and the local reactions were recorded. Each animal was observed once a day for mortality and clinical signs. Body weight was recordedonce during the acclimation period, and thenon days 1 and 6.After 2 days of resting, on day 6, the animals received a single intravenous injection of tritiated methyl thymidine (^{3}H-TdR). Approximately 5 hours later, the animals were sacrificed and the auricular lymph nodes were excised. The proliferation of lymphocytes in the lymph node draining the application site was measured by incorporation of^{3}H-TdR. The results were expressed as disintegrations per minute (dpm) per group and as dpm/node. The obtained values were used to

calculate Stimulation Indices (SI).
No unscheduled deaths and no clinical signs were observed during the observation period.
No local reactions were observed in any animals.
No notable increase in ear thickness was observed at any tested concentrations.
The SI of the positive control was > 3; this experiment was therefore considered valid.
A significant lymphoproliferation was noted at the concentration of 2.5% (SI = 3) and then at the concentrations of 25% and 50% (SI > 3).
In view of the absence of dose-response relationship, it was decided to perform a second counting in order to check the dmp values.
The first results were confirmed. A significant lymphoproliferation (SI > 3) was noted at the concentrations of 2.5, 25 and 50%.
Therefore, despite the absence of dose-response relationship and as no local irritation was observed, the significant lymphoproliferative responses observed were attributed to delayed contact hypersensitivity.
The EC_3value is approximately 2.50%.
Dimethyl disulphideinduced delayed contact hypersensitivity in the murine Local Lymph Node Assay.According to the EC_3value obtained, the test item should be considered as a moderate sensitizer.

[Study 2] WoE.BASF_64V0835/00A033_Skin sensitisation.direct-peptide-binding-assay

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	Supporting study	1 (reliable without restriction)	BASF	2013	In Vitro Sensitization: Direct Peptide Reactivity Assay

Materials and methods

Type of method : in vitro

Type of study : Direct peptide binding assay

Test guideline

Qualifier	**Guideline**	**Deviations**
no guideline available		

Principles of method if other than guideline
Binding to Cys and Lys model peptides in chemico (Bauch C. et al 2011)

GLP compliance : yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.98%

Test system

Traditional sensitisation test

Positive control substance(s) : yes (Ethylene glycol dimethacrylate)

LLNA

Any other information on materials and methods incl. tables

The test substance was prepared as a 100 mM stock solution in acetonitrile just prior to incubation with the synthetic peptides.
Synthetic peptides:
Cysteine- (C-) containing peptide: Ac-RFAACAA-COOH (MW=751.9 g/mol)
Lysine- (K-) containing peptide: Ac-RFAAKAA-COOH (MW=776.2 g/mol)
The peptides are custom material (Supplier: RS Synthesis, Louisville KY, USA) containing phenylalanine to aid in detection and either cysteine or lysine as the reactive center.
Negative control (NC): vehicle control =acetonitrile
Positive control (PC): Ethylene glycol dimethacrylate (EGDMA; CAS-no. 97-90-5) (prepared as a 50 mM solution in acetonitrile)
Because the test-substance preparation was incubated with the peptide shortly after preparation, no analysis of the test substance in the vehicle was performed.
Three samples of the test substance were incubated with each peptide for 24h at room temperature. The incubation tubes were sealed. Additionally triplicates of the concurrent vehicle control (= NC) were incubated with the peptides. The remaining non-depleted peptide concentration was determined thereafter by HPLC with gradient elution and UV-detection at 220 nm. In addition peptide standards of known concentration, prepared from the respective peptide stock solution used for test-substance incubation, were measured in parallel with the same analysis method.
The test substance was incubated with the C-containing peptide in a ratio of 1:10 (0.5 mM peptide, 5 mM test substance, pH 7.5 phosphate buffe
) and with the K-containg peptide in a ratio of 1:50 (0.5 mM peptide, 25 mM test substance, pH 10.2 ammonium acetate buffer).
The mean peptide depletion of a test substance was calculated as the mean value of Cys containing peptide depletion and Lys-containing peptide depletion.
According to the classification tree model described by Gerberick et al. for substances with known molecular weight highly reactive test substance (mean peptide depletion > 42.47 %) is predicted to be a strong sensitizer, a moderately reactive test substance (22.62 % < mean peptide depletion < 42.47 %) a moderate sensitizer, a test substance of low reactivity (6.38 % < mean peptide depletion < 22.62 %) a weak sensitizer. There are no official national or international guidelines for the DPRA Test; however, the study is performed according to the methods described in the following publications:

♣ Gerberick GF, Vassallo JD, Bailey RE, Chaney JG, Morrall SW, Lepoittevin JP. Development of a Peptide Reactivity Assay for Screening Contact Allergens. Toxicological Sciences 81,332-343, 2004.
♣ Gerberick GF, Vassallo JD, Foertsch LM, Price BB, Chaney JG, Lepoittenvin JP. Quantificationn of Chemical Peptide Reactivity for Screening Contact Allergens: A Classification Tree Model Approach. Toxicological Sciences 97(2), 417-427, 2007.
♣ Bauch C, Kolle SN, Fabian E, Pachel C, Ramirez T, Wiench B, Wruck CJ, van Ravenzwaay B, Landsiedel R. Intralaboratory validation of four in vitro assays for the prediction of the skin sensitizing potential of chemicals. Toxicology in Vitro 25, 1162 –
1168, 2011.

Results and discussion

Positive control results

All peptide was depleted by the positive control substance p-benzoquinone.

Traditional sensitisation test

Results of test (except LLNA)

Reading	Hours after challenge	Group	Dose level	No. with + reactions	Total no. in group	Clinical observations
						mean C-peptide depletion with DMDS: 96.8%
						mean K-peptide depletion with DMDS: 0.76%
						mean C-peptide depletion with positive control: 58.4%
						mean K-peptide depletion with positive control: 4.3%

LLNA

Any other information on results incl. tables

The mean C-peptide depletion, caused by the test substance was determined to be 96.80 %.
The mean K-peptide depletion, caused by the test substance was determined to be 0.76 %.
Negative depletions were considered to be "zero" for calculation of the mean peptide depletion, which was thus calculated to be 47.78%.
No co-elution of test substance and peptides was noticed. Adepletion of 36.34 %was caused by the positive control substance.
The test substance was solved in acetonitrile. The samples of the test substance with the peptides were solutions. Visual observation after the 24-hour incubation time did not reveal precipitates in all samples of the test substance with both peptides.
No co-elution of the test substance and peptides occurred as demonstrated by the consistent values of the area ratios 220/258 (lysine-peptide, only) and chromatograms of the co-elution controls.

Applicant's summary and conclusion

Interpretation of results : Peptide binding

[Study 3] WoE.BASF_65V0835/00A034_Skin sensitization.dentritic-cell-activation_MUSST

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Supporting study	1 (reliable without restriction)	BASF	2013	In Vitro Sensitization: Dendritic Cell Line Activation Assay Myeloid U937 Skin Sensitization Test (MUSST)

Materials and methods

Type of method : in vitro

Type of study : Dentritic cell activation assay

Principles of method if other than guideline

CD 86 expression in dentritic cell line as marker for stimulation (Myeloid U937 Skin Sensitization Test, MUSST)

GLP compliance : yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.98%

Test animals

Species : other: U937 cell line (human dentritic-like cell line)

Details on test animals and environmental conditions

Provider of U937 cells: German Resource Center for Biological Material (DSMZ, Germany, catalog no.: ACC 5).

Test system

Traditional sensitisation test

Positive control substance(s) : yes (Ethylene diamine)

LLNA

Any other information on materials and methods incl. tables

Choice of test concentration
A pre-test (experimental conduct in accordance with GLP but without a GLP status) was performed.
Cells were exposed to9 concentrations of the test substance (0.5 µg/mL up to 2000 µg/mL) and cytotoxicity was determined thereafter by propidium iodide (PI) intercalation into the DNA. The CV75 value (= estimated concentration that affords 75% cell viability) was determined by linear regression from the concentration response curve to be 6.23 µg/mL.
In the main test, test substance was used at seven final concentrations determined with regard to the CV75 value: CV75 x 4, CV75 x 2, CV75 x 1.5, CV75 , CV75/2, CV75/4, CV75/8. After 48 hour exposure U937 cells were stained with FITC labeled anti-human-CD 86 antibody and propidium iodide and the fluorescence intensity was analyzed using flow cytometry.
Antibodies:
Antibodies: FITC Mouse anti-human CD86 (BD Pharmingen)
IgG1 FITC CD86 (BD Pharmingen)
Exposure duration: 48h
Vehicle:Culture medium
True negative control: lactic acid 200 µg/mL
Positive control: Ethylene diamine (EDA – 70 µg/mL)
Evaluation criteria
A test substance was predicted to have a dendritic cell activating potential when the marker expression exceeded thethreshold of 1.2with respect to vehicle treated cells (VC) at any tested sufficiently non-cytotoxic (cell viability ≥ 70%) concentration in at least two independent experiments.
Acceptance criteria
If the acceptance criteria mentioned below were not met, repetition of the test was considered. A tested concentration is not to be further evaluated when relative viability is less than 70%. A study is considered acceptable if the positive and negative and vehicle control data lie within the range of the historical data. The cell viability of untreated cells must yield at least 90%. The expression marker CD86 of the vehicle control cells should lie between 8% – 20%.
There are no official national or international guidelines for the MUSST Assay; the study was performed according to the methods described in the following publications:

♣ Python F, Goebel C, Aeby P. (2007) Assessment of the U937 cell line for the detection of contact allergens. Toxicol Appl. Pharmacol. 220(2), 113-24.

♣ Bauch C, Kolle SN, Fabian E, Pachel C, Ramirez T, Wiench B, Wruck CJ, van Ravenzwaay B, Landsiedel R. (2011) Intralaboratory validation of four in vitro assays for the prediction of the skin sensitizing potential of chemicals. Toxicology in Vitro 25,
1162 – 1168.

Results and discussion

Positive control results : Ethylene diamine gave the expected induction of CD86. For details, see table 3

Traditional sensitisation test

Results of test (except LLNA)

Reading	**Hours after challenge**	**Group**	**Dose level**	**No. with + reactions**	**Total no. in group**	**Clinical observations**
						DMDS does induce dendritic cell activation

LLNA

Any other information on results incl. tables

A total of 2 valid experiments were performed (see attached Table 1). The following results were observed:
The test substance was soluble in DMSO (400 x stock solutions) and soluble in 0.25% DMSO (final concentrations).

After 48 hours precipitates were not noticed in any concentration.
In summary, after 48 hours of exposure to test substance Dimethyldisulfide CD86 expression was induced in U937 cells affording at least 70% viability in two independent experiments.
From this it has to be concluded that test substance Dimethyldisulfide does induce dendritic cell activation.

Overall remarks, attachments

Preliminary cytotoxicity assessment:

Table 3

Concentration (test substance) µg/mL	%PI negative cells replicate 1	%PI negative cells replicate 2	%PI negative cells mean	rel. Viability mean
VC	99.1	99.0	99.1	**100.0**
0.5	68.7	96.2	82.4	**83.2**
1	94.1	90.8	92.4	**93.3**
5	79.9	82.5	81.2	**81.9**
10	46.2	55.2	50.7	51.2
50	45.0	44.7	44.8	45.2
100	39.4	43.7	41.5	41.9
500	7.8	44.3	26.1	26.3
1000	1.5	1.4	1.4	1.4
2000	1.9	1.0	1.5	1.5

The CV75 value (= estimated concentration that affords 75% cell viability) was determined by linear regression from the concentration response curve to be 6.23 µg/mL.

Table 1: Summary of Main Experiments. A total of 4 experiments were conducted of which 2 experiments could not be evaluated due to technical faults (experiments 2 and 3, not included in the present report).

Concentration µg/mL	1st experiment		4th experiment	
	CD 86 induction	rel. viability	CD 86 induction	rel. viability
VC	1.00	**100.0**	1.00	**100.0**
0.78	**1.31**	**99.7**	0.46	**100.3**
1.56	**1.86**	**98.5**	1.07	**100.1**
3.12	**2.38**	**92.5**	0.83	**99.0**
6.23	**2.96**	**81.6**	1.04	**91.8**
12.46	**3.40**	53.2	**1.51**	**78.3**

4.2. HISTORICAL CONTROL DATA

Negative Control (Lactic Acid 200 µg/mL)	CD86 MFI	CD86 fold induction	%PI negative cells	rel. viability
Min	4.5	0.56	96.8	98.1
Max	21.7	1.20	99.6	101.4
Mean	13.7	**1.01**	98.9	**100.1**
SD	3.7	0.12	0.6	0.4
n		91		

Positive Control (EDA 70 µg/mL)	CD86 MFI	CD86 fold induction	%PI negative cells	rel. viability
Min	16.8	1.50	61.6	63.7
Max	51.6	4.72	98.9	100.5
Mean	33.2	**2.42**	88.9	**89.9**
SD	8.3	0.54	8.5	8.5
n		91		

Vehicle Control (Medium)	CD86 MFI	CD86 fold induction	%PI negative cells	rel. viability
Min	5.3		96.8	
Max	19.9		99.5	
Mean	12.9	**1.0**	98.8	**100.0**
SD	3.7		0.6	
n		46		

Vehicle Control (DMSO)	CD86 MFI	CD86 fold induction	%PI negative cells	rel. viability
Min	8.2		97.0	
Max	19.9		99.5	
Mean	14.4	**1.0**	98.8	**100.0**
SD	3.1		0.7	
n		40		

Applicant's summary and conclusion

Interpretation of results : activation of dentritic cells in vitro

[Study 4] WoE.BASF_66V0835/00A035_Skin sensitisation.Lu-Sens

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Supporting study	1 (reliable without restriction)	BASF	2013	ARE Reporter Assay - LuSens

Materials and methods

Type of method : in vitro

Type of study : other: in-vitro keratinocyte activation assay (LuSens)

Principles of method if other than guideline

The LuSens assay is an in vitro method for the identification of keratinocyte activating substances using the genetically modified keratinocytes (LuSens, Bauch et al. 2012). lt employs the reporter gene for luciferase under the control of an antioxidant response element and hence monitors Nrf-2 transcription factor activity. The endpoint measurement is the up-regulation of the luciferase activity after 48 hour incubation with test substances. This up-regulation is an indicator for the activation of the Keap1/Nrf2/ARE signaling pathway (Ade et al. 2009, Natsch 2012, Natsch & Emter 2008, Vandebriel et al. 2010).

GLP compliance : yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Purity: 99.98%

Test animals

Species : other: LuSens cells (derived from human keratinocyte cell line HaCaT)

Details on test animals and environmental conditions

The reporter gene cell line LuSens was prepared in collaboration with the RWTH Aachen University. This keratinocyte cell line derived from HaCaT cells carries a reporter gene for luciferase under the control of an antioxidant-response-element (ARE) and hence monitors Nrf2 transcription factor activity. The ARE promoter belongs to the NADPH:quinone oxidoreductase1 gene from rats.

Test system

Traditional sensitisation test

Positive control substance(s): yes (Ethylene glycol dimethacrylate)

LLNA

Any other information on materials and methods incl. tables

LuSens cells were cultured in complete DMEM culture medium with high glucose supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin – 100 lg/mL streptomycin and 0.5 lg/mL puromycin in T75 culture flasks. Cells were

grown for 24 h in 96-well plates prior to the substance treatment (10000 cells in 0.12 mL medium). Stock solutions of the substances were prepared by dissolving in DMSO (final concentration of 200 mM) and diluted in 2-fold dilutions. Test substance solutions were further diluted in medium containing 1% FBS w/o puromycin to obtain a DMSO concentration of 1%.
The highest tested concentration in the main experiment was 1.22 fold of the concentration affording a viability of 75% (CV75). The additional concentrations were obtained by a 1:1exp2 dilution series of the CV75.
Cytotoxicity was determined via the MTT assay.
Two independent experiments were performed. In each experiment, three duplicates of each treatment were tested.
Positive control (PC): Ethylene glycol dimethacrylate (EGDMA 18 µg/ml)
Negative control (NC): DL-Lactic acid (LA 450 µg/mL)
Vehicle control: 1% DMSO in culture medium
Substance incubation was performed under standard cell culture conditions for 48 h and luciferase activity then determined using SteadyGlo™ (Promega, Germany) according to manufacturer's instructions.
Acceptance criteria
A tested concentration was not further evaluated when relative viability is less than 70%.
The cell viability of untreated cells must yield at least 90%. The positive control EGDMA should be ~3 fold induction and lactic acid <2 and viability ~70%. The average standard of the variability in the vehicle control wells for each plate should be below 20%.
A study is considered acceptable if the positive and negative and vehicle control data lies within the range of the historical data.
Evaluation criteria
A test substance is concluded to exhibit an keratinocyte activating potential when the luciferase activity exceeds 2.0 fold induction with respect to the vehicle control and at concentrations that do not reduce a viability below 70%.

Results and discussion

Traditional sensitisation test

Results of test (except LLNA)

Reading	Hours after challenge	Group	Dose level	No. with + reactions	Total no. in group	Clinical observations
						DMDS had no effect on the luciferase activity in LuSens cells

LLNA

Any other information on results incl. tables

After 48 hours of exposure to test substance Dimethyldisulfide luciferase activity in LuSens cells was not induced affording at least 70% viability in at least two independent experiments (see attached Table 1). From this it has to be concluded that test substance Dimethyldisulfide does not have a keratinocyte activating potential.

Overall remarks, attachments

Table 1: Summary of Main Experiments

Concentration µg/mL	1st experiment fold induction mean	SD	rel. viability [%] mean	SD	2nd experiment fold induction mean	SD	rel. viability [%] mean	SD
VC	1.00	0.27	**100.0**	2.7	1.00	0.17	**100.0**	2.7
8.85	0.76	0.16	**98.7**	2.2	n.d.	n.d.	n.d.	n.d.
10.62	0.75	0.38	**99.3**	2.3	n.d.	n.d.	n.d.	n.d.
12.74	0.75	0.09	**99.8**	4.2	n.d.	n.d.	n.d.	n.d.
15.29	0.84	0.15	**97.0**	1.5	0.87	0.26	**97.0**	10.5
18.35	0.91	0.09	**94.8**	1.4	1.07	0.25	**87.9**	3.3
22.02	0.84	0.15	**94.8**	5.1	0.97	0.05	**93.1**	5.4
26.43	0.90	0.27	**91.8**	2.6	1.04	0.17	**89.3**	2.4
31.71	0.81	0.21	**84.9**	3.1	1.09	0.29	**84.5**	1.9
38.05	n.d.	n.d.	n.d.	n.d.	1.01	0.12	**78.1**	3.5
45.66	n.d.	n.d.	n.d.	n.d.	0.99	0.05	**72.3**	2.5
54.79	n.d.	n.d.	n.d.	n.d.	1.07	0.17	30.6	5.1
EGDMA (18 µg/mL)	**4.19**	0.49	**94.2**	2.3	**3.86**	0.49	**101.4**	2.4
LA (450 µg/mL)	1.38	0.34	**105.6**	2.1	0.90	0.16	**103.8**	2.9

Applicant's summary and conclusion

Interpretation of results : no induction of antioxidant response genes in a keratinocyte cell line

3.8 Repeated dose toxicity – Inhalation route

[Study 1] Collins (Hazleton-UK) 1992/K1 KS/90-d inhalation toxicity

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key Stduy	1 (reliable without restriction)	Collins CJ	1992	DMDS: 90 day inhalation toxicity study in the rat with a 4 week recovery period

Materials and methods

Test type : subchronic

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)	

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.88%

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : male/female

Details on test animals and environmental conditions

TEST ORGANISMS:
- Source: Charles River UK Ltd., Margate
- Age at reception: 4-6 weeks
- Weight at reception: 120-140 g for males, 80-100 g for females
- Weight at the start of the treatment: 185-256 g for males, 121-169 g for females
- Number of animals: 100 rats : 20 males + 20 females / dose group (4 dose groups + 1 control group)
- Acclimatation period: 14 days

HOUSING
The animals were housed in group of 5 in suspended stainless steel cages.

FOOD and WATER
- Food: SZQC rat and Mouse Maintenance Diet No. 1 ad libitum excepted during exposure
- Water: filtered tap water, ad libitum excepted during exposure

ENVIRONMENTAL CONDITIONS
- Temperature : 19-25°C
- Relative humidity : 40-70%
- Light/dark cycle : 12h/12h
- Ventilation : 15 air changes/hour

Administration / exposure

Route of administration : inhalation

Type of inhalation exposure : whole body

Vehicle : other: unchanged (no vehicle)

Details on inhalation exposure

Five horizontal flow, recirculating exposure chambers were used. Each was made of stainless steel with perspex (Plexiglas) doors and a fan to mix the atmospheres by recirculation. The compressed air supply was from a clean, dry, filtered source. The total volume of the animals did not exceed 5% of the volume of the test chamber. The four concentrations of test article vapour were produced by passing metered flows of air through sintered glass frits immersed in separate containers of test article. The resulting outputs of vapour were introduced to the diluent air inlet duct of each test chamber. Mixing, within the duct and recirculation system, ensured the production of homogeneous atmospheres for animal exposure. The chambers were ventilated at a rate of at least 12 air changes per hour. Air flows were monitored continuously and recorded twice hourly during exposure. The exhaust streams were purified with activated charcoal and vented to the outside of the building.

Analytical verification of doses or concentrations : yes

Details on analytical verification of doses or concentrations

* Measured concentration Samples for analysis were withdrawn from the exposure chambers twice hourly through sample lines leading from each chamber through a sampling valve into a total hydrocarbon analyser. The analysis was performed with an Analysis Automation total hydrocarbon analyser type 523 Detector with a Flame ionisation detector (FID)
* Nominal concentration The total weight of test article used and total volume of diluent air were measured for each exposure.
- Group Target Nominal Analytical concentrations:

1	0		
2	10	20	10.17 ppm
3	50	81	50.25 ppm
4	150	223	150.62 ppm
5	250	373	246.59 ppm

Duration of treatment / exposure : 90 days

Frequency of treatment : 6 h/day; 5 d/week

Doses/concentrations : 10, 50, 150, 250 ppm

Basis analytical conc.

MMAD / GSD

A simslin II dust monitor was used pre-dose, and during the study at week 1, 4, 8, and 12, at each exposuire levels to confirm all the test article was in a vapour phase.

No. of animals per sex per dose : 20

Control animals : yes, concurrent vehicle

Details on study design

Four groups of 20 male and 20 female Sprague-Dawley were exposed 6 hours/day, 5 days/week to 0, 10, 50, 150, or 250 ppm DMDS. The exposure of the 150 ppm group was terminated after 6 weeks and its treatment-free subgroup necropsied 2 weeks later. The remaining groups received a 13 week exposure period followed by four weeks for the treatment-free subgroups.

Positive control : Not appropriate

<u>Examinations</u>

Observations and examinations performed and frequency

- Clinical observations
* Morbidity and mortality All animals were examined twice daily to detect any which were dead or moribund.
* Clinical signs All animals were examined once daily for signs of ill health or overt toxicity. In addition each animal was given a detailed clinical examination at weekly intervals. An individual record was maintained of the clinical condition of each animal.
* Functional observation tests Observations were carried out on all animals prior to beginning treatment and again during weeks 1, 4, 13 and the treatment-free animals in week 17. The observations were made prior to and following any exposure that day. Observations were recorded for the following parameters: ease of removal from home cage, ease of handling, appearance of eyelids, lacrimation, colour of tears, salivation, respiration, appearance of fur, piloerection, writhing, vocalisation.
- Body weight Individual body weights were recorded before exposure on the first day of the study, at weekly intervals thereafter and at necropsy.
- Food consumption The amount of food consumed by each cage of animals was determined weekly.
- Ophthalmoscopy The eyes of all animals were examined pre-dose and all control and high dose animals in week 12.
- Laboratory investigations Blood samples were obtained from groups 1, 4 and 5 for haematology and clinical chemistry in week 6 and groups 1 and 5 for haematology and clinical chemistry in week 12. The samples were collected from the main study animals.
* Haematology: Haemoglobin, mean cell volume, red blood cell count and indices: mean cell haemoglobin, mean cell haemoglobin concentration packed cell volume, total and differential white blood cell count platelet count.
* Clinical chemistry: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, sodium, potassium, chloride, calcium inorganic phosphorus, glucose, urea, total bilirubin, creatinine, total protein, albumin, albumin/globulin ratio total cholesterol.

Sacrifice and pathology

- Pathology
* Necropsy: Full internal and external examination at sacrifice

* Organ weights: adrenals, brain, kidneys, liver, lungs, ovaries, pituitary, spleen, testes.
* Histology: adrenals, femur (including articular aorta surface)#, brain (including brain stem), heart, caecum, ileum colon, jejunum duodenum, kidneys, epididymides#, lachrymal gland#, eyes (with optic nerves), larynx, liver, sciatic nerve, lungs (with mainstem bronchi), skeletal muscle (quadriceps)#, lymph nodes (bronchial with tracheal bifurcation and mesenteric), skin and mammary gland#, spinal cord (lumbar, cervical, thoracic)#, nasal passages, spleen, nasopharyngeal duct, sternum (and bone marrow), oesophagus, stomach, ovaries, testes, pancreas, thymus, pituitary, thyroids (with parathyroids), prostate#, tracheal bifurcation, rectum, urinary bladder, salivary glands (submaxillary, sublingual), uterus and all gross lesions. Samples of all tissues (except those annotated # above) from all main study animals in the control and high dose group, the lungs and all gross lesions of all main study animals and the nasal cavities of groups 2 and 3 terminal kill and groups 1, 2, 3 and 5 treatment-free animals were evaluated by the study pathologist.

Statistics

* ANOVA, T-test Body weight: week 0
* ANOVA, Regression and Dunnett's Body weight gains: weeks 0 to 6, 0 to 13, 13 to 17 weeks 6 to 8 (group 1 v group 4 only) Total food consumption: weeks 1 to 5, 7 to 12 Necropsy body weight: terminal kill and treatment-free Clinical chemistry: weeks 6, 12 AST, ALT, ALK PROS, Na, K, Cl, Ca, P, GLUCOSE, UREA, T BILI, CREAT, T PROT, ALBUMIN, AG RATIO, TOT CHOL Clinical chemistry: week 13 ALT, ALK PHOS (males), T BILI Clinical chemistry: week 17 ALT, ALK PHOS, T BILI Haematology: weeks 6, 12 Hb, RBC, PCV, MCV, MCH, MCHC, PLAT, WBC, Neutrophils and Lymphocytes - absolute and percentages Haematology: week 13 Hb, RBC, PCV, MCV, MCH, MCHC
* ANCOVA, Dunnett's Organ weights (adjusted for necropsy body weight) - terminal kill and treatment-free: adrenals, kidneys, spleen, liver, ovaries, gonads, lung, brain, pituitary
* Kruskal-Wallis, Terpstra-Jonckheere, Wilcoxon Clinical chemistryweek 13 females ALK PHOS No statistical analysis was considered necessary to interpret the results of the functional observation tests.

<u>Results and discussions</u>

Effect levels

Endpoint	**Effect level**	**Based on**	**Sex**	**Basis for effect level / Remarks**
NOAEC (Systemic toxicity)	10 ppm (analytical)		male/female	
LOAEC (Systemic toxicity)	50 ppm (analytical)	test mat.	male/female	Clinical signs of toxicity (soiled fur and piloerection); decreased body weights (↓2-14%), body weight gains (↓17-36%), and food consumption (↓4-15%) in both sexes.
LOAEC (Nasal irritation)	10 ppm (analytical)	test mat.	male/female	Minimal respiratory squamous metaplasia in 3/10 males and 4/10 females

<u>Results of examinations</u>

Clinical signs and mortality : yes

Body weight and weight gain : yes

Food consumption : no effects

Food efficiency : no data

Water consumption : not examined

Ophthalmoscopic examination : no effects

Haematology : yes

Clinical chemistry : yes

Urinalysis : not examined

Neurobehaviour : yes

Organ weights : no effects

Gross pathology : no effects

Histopathology: non-neoplastic : yes

Histopathology: neoplastic : not examined

Details on results

MORTALITY
There was no treatment-related mortality.
CLINICAL SIGNS
The only clinical signs attributable to treatment were salivation, lacrimation or reduced activity during exposure 1 and 2 of the 150 and 250 ppm groups and a low incidence of dyspnoea or wheezing in the early part of the study, particularly in the 250 ppm animals at week 1. FOB Functional observation tests indicated treatment-related changes in response to handling and respiration, and in incidence of salivation, soiling of the fur and piloerection but no evidence of neurotoxicity.
BOBY WEIGHT There was a dosage-related decrease in body weight gain (Tables 1 and 2) over the treatment period in treated groups compared with controls. Differences were statistically significant except for the 10 ppm group which was only significant for males over weeks 0 to 6. Improvements in the rate of body weight gain were observed in the test groups in the treatment-free period.
FOOD CONSUMPTION
Differences in food consumption (Table 3) paralleled those of body weight gain except the numerical differences did not achieve statistical significance in the 50 ppm males or the 10 ppm groups.
OPHTHALMOSCOPY The eyes of the animals were unremarkable.
HAEMATOLOGY
No effect was observed on the haematological profiles of the males on week 6 (0, 150 and 250 ppm groups). In females, a slight statistically significant decrease of MCV (58.7 vs 60.4 fl) and MCH (20.1 vs. 21.1 pg) was observed at 150 ppm and of MCHC (34.1 vs. 34.8 g/dl) at 250 ppm when compared to the control group.Haematological profiles on week 12 (0 and 250 ppm groups, Table 4)) suggested a possible small reduction in Hb, RBC and PCV in the 250 ppm female group only. Haematological profiles on week 13 (0, 10 and 50 ppm groups, Table 5) were unremarkable (data not shown)
BLOOD CHEMISTRY
Blood chemistry examinations performed on week 6 (0, 150 and 250 ppm groups) showed in males a statistically significant increases of ALT (62 vs. 45 Iu/l) at 150 ppm and Alk. Phos. (450 vs. 338 Iu/l) and total bilirubin (5.2 vs. 3.9 µmol/l) at 250 ppm when compared to the control group. In females, significant increases of total bilirubin (4.1 vs. 3.1 µmol/l) was observed at 150 ppm and of alk. phos. (329 vs. 242 Iu/l) at 250 ppm. A decrease of the urea level was also observed at 150 ppm (6.6 vs. 7.7 mmol/l).Blood chemistry examinations performed on week 12 (0 and 250 ppm groups, Table 6) and 13 (0, 10 and 50 ppm groups, Table 7) showed treatment-related changes in ALT, alkaline phosphatase and bilirubin. The changes did not include the 10 ppm group except for elevated ALT in occasional animals at week 13 and after the treatment-free period. Any changes in alkaline phosphatase and bilirubin in the 50 ppm group were equivocal. Blood chemistry examinations performed on week 17 (0, 10, 50 and 250 ppm groups) did not show any treatment related effects on ALT, alk. phos. and total bilirubin.
ORGAN WEIGHTS
There were no changes in organ weights that were considered to be treatment-related. Statistically significant elevations in lung weights in the 250 ppm males and females were of doubtful toxicological importance.
MACROSCOPIC OBSERVATIONS
There were no treatment-related macroscopic abnormalities at necropsy.
MICROSCOPIC OBSERVATIONS
In the 10, 50 and 250 ppm animals examined microscopically there was a dose-related effect on nasal mucosa characterised by squamous metaplasia of the respiratory epithelium accompanied in 50 ppm and 250 ppm groups by atrophy and microcavitation in the anterior olfactory epithelium. In the 10 ppm group the effects were limited to a local, minor degree of squamous metaplasia of the anterior nasal cavity. The changes were still present in the 50 and 250 ppm groups after the treatment-free period but the 10 ppm group was generally unremarkable.

Any other information on results incl. tables

Table 1: Group mean body weight gains (g), males

Week of study	Concentration (ppm)

		0	10	50	150	250
Start	Mean	227.3	224.5	228.6	225.2	227.1
	S.D.	15.69	13.15	13.36	14.36	14.82
0 to 6	Mean	160.2	139.0*	128.7**	115.2***	106.3***
	S.D.	31.86	24.03	28.84	24.99	21.50
6 to 8	Mean	32.5-	-	-	47.5***	-
	S.D.	10.67	-	-	8.68	-
0 to 13	Mean	233.0	225.6	181.8***	-	148.3***
	S.D.	45.31	34.38	42.47	-	37.83
13 to 17	Mean	10.8	25.9	29.5	-	41.8**
	S.D.	18.17	23.94	17.62	-	21.01

* p<0.05** p<0.01*** p<0.001

Table 2: Group mean body weight gains (g), females

Week of study		Concentration (ppm)				
		0	10	50	150	250
Start	Mean	149.6	146.0	144.9	145.6	144.2
	S.D.	7.53	8.62	5.47	10.88	10.9
0 to 6	Mean	88.0	77.0	73.0**	68.1***	74.2**
	S.D.	14.97	15.00	10.72	15.86	13.51
6 to 8	Mean	14.8	-	-	20.3*	-
	S.D.	5.22	-	-	5.30	-
0 to 13	Mean	117.8	119.8	97.5**	-	93.2***
	S.D.	22.69	22.63	17.13	-	17.99
13 to 17	Mean	3.0	5.5	9.5	-	9.6
	S.D.	8.86	12.91	11.08	-	11.67

* p<0.05** p<0.01*** p<0.001

Table 3:mean food consumption (g/animal) over specified periods

Week of study		Concentration (ppm)				
		0	10	50	150	250
Males						
1 to 5	Mean	810.2	779.4	752.3	772.4*	700.4**
	S.D.	33.85	46.25	36.88	40.86	18.17
7 to 12	Mean	991.7	996.1	944.0	—	866.8***
	S.D.	12.46	42.34	39.49	—	24.38
Females						
1 to 5	Mean	585.5	558.5	527.5**	516.1**	535.6*
	S.D.	17.77	19.63	14.23	31.86	19.34
7 to 12	Mean	741.1	728.4	674.2***	—	691.1**
	S.D.	3.54	27.29	9.63	—	21.2

* p<0.05** p<0.01*** p<0.001

Table 4: Group mean haematology, Occasion: Week 12

Group		Hb	RBC	PCV	MCV	MCH	MCHC	PLAT
Sex		g/dl	mil/cmm	%	fl	pg	g/dl	1000/cmm
Males								
0 ppm	Mean	15.0	8.16	45.9	56.4	18.4	32.6	926
	S.D.	0.7	0.56	1.9	2.0	0.7	0.4	147
250 ppm	Mean	14.4	7.76	44.0	56.7	18.5	32.7	936
	S.D.	0.8	0.44	2.5	1.3	0.5	0.3	128
Females								
0 ppm	Mean	14.4	7.44	43.4	58.4	19.4	33.2	967
	S.D.	0.7	0.34	2.1	1.5	0.6	0.3	140
250 ppm	Mean	13.5*	6.88**	41.0*	59.6	19.7	33.0	947
	S.D.	0.8	0.42	2.3	1.7	0.6	0.4	73

* p<0.05 ** p<0.01 *** p<0.001

Table 5: Group mean haematology, Occasion: Week 12

Group Sex		WBC 1000 / cmm (%) TOTAL	N	L	M	E	B
Males							
0 ppm	Mean	13.2	1.71(13)	11.23(85)	0.12(1)	0.12(1)	0.00(0)
	S.D.	4.5	0.71(-)	3.91(-)	0.13(-)	0.14(-)	0.00(-)
250 ppm	Mean	11.7	1.31(11)	10.33(88)	0.01(0)	0.04(0)	0.00(0)
	S.D.	3.5	0.50(-)	3.23(-)	0.04(-)	0.06(-)	0.00(-)
Females							
0 ppm	Mean	11.0	1.25(12)	9.22(84)	0.28(3)	0.20(2)	0.00(0)
	S.D.	2.7	0.82(-)	2.51(-)	0.15(-)	0.23(-)	0.00(-)
250 ppm	Mean	10.8	1.30(12)	9.13(85)	0.28(3)	0.10(1)	0.00(0)
	S.D.	1.7	0.43(-)	1.45(-)	0.18(-)	0.08(-)	0.00(-)

Table 6: Group meanclinicalchemistry, Occasion: Week 12

Group Sex		AST(GOT) Iu/l	ALT(GPT) Iu/l	ALKPHOS Iu/l	Na mmol/l	K mmol/l	Cl mmol/l	Ca mmol/l	P mmol/l
Males									
0 ppm	Mean	96	51	222	143	3.9	108	2.41	1.8
	S.D.	13	11	50	1	0.2	2	0.10	0.1
250 ppm	Mean	78**	47	302*	143	3.8	106	2.44	1.8
	S.D.	9	7	82	1	0.3	2	0.09	0.1
Females									
0 ppm	Mean	86	39	168	141	3.4	107	2.46	1.7
	S.D.	9	6	37	1	0.2	1	0.09	0.1
250 ppm	Mean	84	51*	264*	139*	3.4	106	2.38*	1.7
	S.D.	9	14	108	2	0.3	3	0.06	0.1

* p<0.05 ** p<0.01*** p<0.001

Group Sex		GLUC mmol/l	UREA mmol/l	T BILI µmol/l	CREAT µmol/l	T PROT g/l	ALBUMIN g/l	AG RATIO	TOT CHOL mol/l
Males									
0 ppm	Mean	5.9	6.0	3.7	62	62	34	1.3	1.7
	S.D.	0.7	1.0	0.6	3	4	1	0.1	0.3
250 ppm	Mean	6.0	6.1	4.8*	59*	62	36*	1.4	1.5
	S.D.	0.5	0.9	1.2	2	3	1	0.1	0.3
Females									
0 ppm	Mean	6.2	7.0	3.5	65	68	40	1.4	2.2
	S.D.	0.3	0.9	0.6	5	3	2	0.1	0.4
250 ppm	Mean	6.1	6.7	5.7***	65	68	39	1.4	2.1
	S.D.	0.6	1.1	1.1	2	5	1	0.2	0.4

* p<0.05 ** p<0.01*** p<0.001

Table 7: Group mean clinicalchemistry, Occasion: Week 13

Group Sex		ALT(GPT) Iu/l	ALK PHOS Iu/l	T BILI µmol/l
Males				
0 ppm	Mean	48	282	2.0
	S.D.	5	45	1.0
10 ppm	Mean	61	345	2.5
	S.D.	16	112	0.6
50 ppm	Mean	72*	375	2.2
	S.D.	35	101	0.6
Females				
0 ppm	Mean	46	218	1.2
	S.D.	8	59	0.5

10 ppm	Mean	75	264	1.3
	S.D.	42	47	0.5
50 ppm	Mean	57*	320	1.4
	S.D.	10	128	0.9

* p<0.05 ** p<0.01*** p<0.001

TABLE 8.2

Group mean organ weights (g) adjusted to mean necropsy body weight Terminal kill (week 14)

Group and sex	Bodyweight g	Adrenals	Kidney	Spleen	Liver
1M	420.3	.055 (.057)	2.137 (2.351)	.667 (.706)	8.782 (9.591)
2M	406.8	.054 (.055)	2.189 (2.314)	.703 (.726)	9.295 (9.767)
3M	388.0	.055 (.055)	2.308 (2.310)	.655 (.655)	9.282 (9.260)
5M	335.7***	.049 (.046)	2.445* (2.105)	.688 (.626)	9.349 (8.060)
1F	244.9	.049 (.051)	1.449 (1.495)	.445 (.463)	6.145 (6.406)
2F	244.9	.056 (.058)	1.488 (1.534)	.455 (.474)	6.317 (6.577)
3F	222.6	.064* (.063)	1.489 (1.454)	.466 (.452)	6.593 (6.391)
5F	216.9*	.061 (.059)	1.498 (1.442)	.512* (.489)	6.572 (6.253)

( ) = absolute means
* p<0.05
** p<0.01
*** p<0.001

TABLE 8.2

Group mean organ weights (g) adjusted to mean necropsy body weight Terminal kill (week 14)

Group and sex	Bodyweight g	Gonads	Lung	Brain	Pituitary
1M	420.3	3.301 (3.342)	1.372 (1.476)	2.051 (2.082)	0.013 (0.014)
2M	406.8	3.263 (3.287)	1.425 (1.486)	2.050 (2.068)	0.013 (0.014)
3M	388.0	3.449 (3.449)	1.484 (1.485)	2.039 (2.040)	0.014 (0.014)
5M	335.7***	3.354 (3.289)	1.534* (1.368)	2.041 (1.992)	0.013 (0.011)
1F	244.9	.066 (.068)	1.075 (1.126)	1.899 (1.919)	.017 (.017)
2F	244.9	.066 (.068)	1.076 (1.127)	1.857 (1.878)	.017 (.017)
3F	222.6	.069 (.068)	1.142 (1.102)	1.914 (1.898)	.018 (.018)
5F	216.9*	.078 (.076)	1.195* (1.132)	1.882 (1.857)	.015 (.015)

( ) = absolute means
* p<0.05
** p<0.01
*** p<0.001

TABLE 8.2

Group mean organ weights (g) adjusted to mean necropsy body weight
Treatment-free kill (week 18)

Group and sex	Body weight (g)	Adrenals	Kidney	Spleen	Liver
1M	465.9	.053 (.056)	2.286 (2.393)	.711 (.763)	9.653 (10.291)
2M	470.3	.048 (.052)	2.274 (2.401)	.643 (.705)	9.861 (10.619)
3M	422.8	.052 (.050)	2.449 (2.360)	.720 (.677)	10.765* (10.232)
5M	408.7 DR**	.058 (.054)	2.408 (2.255)	.853* (.778)	11.634*** (10.718)
1F	263.5	.058 (.060)	1.436 (1.510)	.410 (.431)	6.207 (6.528)
2F	258.1	.060 (.062)	1.542 (1.592)	.458 (.472)	6.490 (6.705)
3F	236.1*	.058 (.057)	1.479 (1.429)	.408 (.393)	6.458 (6.241)
5F	230.9**	.065 (.062)	1.522 (1.448)	.478* (.457)	6.480 (6.161)

( ) = absolute means
* p<0.05
** p<0.01
*** p<0.001
DR = significant using a dose-response test

TABLE 8.2

Group mean organ weights (g) adjusted to
mean necropsy body weight
Treatment-free kil[week 18)

Group and sex	Body weight (g)	Gonads	Lung	Brain	Pituitary
1M	465.9	3.343 (3.429)	1.500 (1.559)	2.029 (2.041)	0.011 (0.012)
2M	470.3	3.475 (3.578)	1.453 (1.523)	2.090 (2.104)	0.012 (0.013)
3M	422.8	3.634 (3.562)	1.557 (1.508)	2.101 (2.091)	0.014 (0.013)
5M	408.7 DR**	3.494 (3.370)	1.615 (1.531)	2.090 2.073	0.014 0.013
1F	263.5	≠ (0.073)	1.184 (1.233)	1.910 (1.922)	0.019 (0.019)
2F	258.1	≠ (0.077)	1.141 (1.175)	1.911 (1.919)	0.017 (0.018)
3F	236.1*	≠ (0.071)	1.138 (1.108)	1.912 (1.904)	0.018 (0.018)
5F	230.9**	≠ (0.081)**	1.194 (1.137)	1.866 (1.854)	0.015 (0.014)

( ) = absolute means
* p<0.05
** p<0.01
*** p<0.001
DR = significant using a dose-response test
≠ value not calculable due to heterogeneity of slopes

Text table 1: Incidence of selected histopathology findings in nasal cavity - terminal kill (week 14)

Finding - Grade	1M	2M	3M	5M	1F	2F	3F	5F
	Group and sex							
Respiratory squamous metaplasia								
- not detected	10	7	0	0	10	6	0	0
- minimal	0	3	4	0	0	4	5	0
- slight	0	0	6	9	0	0	5	10
- moderate	0	0	0	1	0	0	0	0
Olfactory epithelial atrophy/ microcavitation								
- not detected	10	10	2	0	10	10	2	0
- minimal	0	0	7	0	0	0	8	0
- slight	0	0	1	2	0	0	0	4
- moderate	0	0	0	8	0	0	0	6
No. of animals/group	10	10	10	10	10	10	10	10

Text table 2: Incidence of selected histopathology findings in nasal cavity - treatment-free kill, week 18

Finding - Grade	1M	2M	3M	5M	1F	2F	3F	5F
	Group and sex							
Respiratory squamous metaplasia								
- not detected	10	10	5	0	10	10	4	0
- minimal	0	0	4	1	0	0	6	0
- slight	0	0	0	9	0	0	0	10
Olfactory epithelial atrophy/ microcavitation								
- not detected	10	9	1	0	10	10	0	0
- minimal	0	1	8	1	0	0	10	1
- slight	0	0	0	9	0	0	0	7
- moderate	0	0	0	0	0	0	0	2
No. of animals/group	10	10	9	10	10	10	10	10

Applicant's summary and conclusion

Conclusions

Clear treatment-related effects were seen at 50 and 250 ppm and were present to a marginal degree at 10 ppm. It was concluded that the effect level was 50 ppm. The no-effect level was in the region of, but less than, 10 ppm due to the reversible changes in the nasal mucosa

Executive summary

In an OECD 413 study, groups of 10 rats/sex were exposed by inhalation to DMDS 6 h/day, 5 d/week for 90 days to concentrations of 0, 10, 50, 150, 250 ppm (Collins, 1992). The exposure of the 150 ppm group was terminated after 6 weeks and its treatment-free subgroup necropsied 2 weeks later. The remaining groups received a 13 week exposure period followed by 4 weeks for the treatment-free subgroups. The only clinical signs attributable to treatment were salivation, lacrimation or reduced activity during exposures 1 and 2 of the 150 and 250 ppm groups and a low incidence of dyspnea or wheezing in the early part of the study, particularly in the 250 ppm animals at week 1. Functional observation tests indicated no evidence of neurotoxicity. Body weight gains and food consumption were decreased in all treatment groups; this effect was reversible during the recovery period. Hematological profiles suggested a possible small reduction in Hb, RBC and PCV in the 250 ppm female group only. Blood chemistry examinations showed treatment-related changes in ALT, alkaline phosphatase and bilirubin. The changes did not include the 10 ppm group except for elevated ALT in occasional animals at week 13 and after the treatment-free period. There were no changes in organ weights that were considered to be treatment-related and no treatment-related macroscopic abnormalities. Microscopic evaluations indicated a dose-related effect on nasal mucosa characterised by squamous metaplasia of the respiratory epithelium accompanied by atrophy and microcavitation in the anterior olfactory epithelium. In the 10 ppm group the effects were limited to a local, minor degree of squamous metaplasia of the anterior nasal cavity. The changes were still present in the 50 and 250 ppm groups after the treatment-free period but the 10 ppm group was generally unremarkable. Clear treatment-related effects were seen at 50 and 250 ppm and were present to a marginal degree at 10 ppm.

[Study 2] Kim et al., 2006/K1 KS/90-d inhalation toxicity

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title	Bibliographic source
publication	Key Stduy	1 (reliable without restriction)	Kim HY, Lee SB, Chung YH, Lim CH and Yu IJ	2006	Evaluation of Subchronic Inhalation Toxicity of Dimethyl Disulfide in Rats	Inhalation Toxicology, 18:395–403.
Other	Key Stduy	1 (reliable without restriction)	Kim JC	2009	Evaluation of Subchronic Inhalation Toxicity of Dimethyl Disulfide in Rats	

Materials and methods

Test type : subchronic

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)	

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: >= 99% (Merck 's specification)

Test animals

Species : rat

Strain : Fischer 344

Sex : male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Japan Inc. (Kanagawa, Japan)
- Age at study initiation: 6-wk-old
- Weight at study initiation: no data
- Fasting period before study: not appropriate
- Housing: individually in wire-bottomed stainless steel wire mesh cages
- Diet : commercial rodent chow (LabDiet 5002, PMI Nutrition, USA), ad libitum.
- Water: sterilized tap water, ad libitum
- Acclimation period: 1 week

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 23 +/- 3°C
- Humidity (%): 50 +/- 10%
- Air changes (per hr): 12-15
- Photoperiod (hrs dark / hrs light): 12/12 hrs

IN-LIFE DATES: no data

Administration / exposure

Route of administration : inhalation: vapour

Type of inhalation exposure : whole body

Vehicle : other: unchanged (no vehicle)

Details on inhalation exposure

GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION
- Exposure apparatus: stainlesssteel chamber (1000 L).- Method of holding animals in test chamber: no data- Source and rate of air: no data- Method of conditioning air: no data
- System of generating particulates/aerosols: not appropriate- Temperature and humidity in air chamber: 22.1-25.7°C, 45.2-59.6%- Air flow rate: no data- Air change rate: no data
- Method of particle size determination: no data
- Treatment of exhaust air: no data

TEST ATMOSPHERE
- Brief description of analytical method used: Gas chromatography (Shimadzu Co., Japan) : detector, flame ionization detector; column, silicon DC-200 15% chromosorb with mesh of 80/100 and a 0.5 m length; detector temperature, 200°C; oven temperature, 100°C; injector temperature, 200°C; and injection volume, 1 ml of gas sample.
- Samples taken from breathing zone: no data

Analytical verification of doses or concentrations : yes

Details on analytical verification of doses or concentrations

DMDS vapor concentrations in the chambers during exposure were measured every 15 min and were controlled to be within ±6% of the target concentration using a personal computer. The mean concentration measured every 15 min for 6

h was taken as the value on a given day. This was then averaged over the 13-wk exposure in order to obtain the mean and standard deviations, and the daily gas concentrations in the 3 chambers were measured at 5.3 ± 0.16, 25.3 ± 0.43, and 125.0 ± 1.34 ppm, respectively.

Duration of treatment / exposure : 13 weeks

Frequency of treatment : 6 h/day, 5 days/wk

Doses/concentrations : 5, 25, or 125 ppm

Basis analytical conc.

No. of animals per sex per dose : 10

Control animals : yes, sham-exposed

Details on study design

- Dose selection rationale: The experimental concentrations were selected based on the results of a preliminary dose-range-finding study. Groups of 5 rats of each gender were exposed to DMDS via whole-body inhalation at concentrations of 33, 100, and 300 ppm for 2 wk. Males at 300 ppm and females at above 33 ppm showed reduced body weight gain and/or decreased food intake.
- Post-exposure recovery period in satellite groups: none

Positive control :Not appropriate

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes
All animals were observed twice daily (before and after exposure) throughout the study period for any clinical signs of toxicity, moribundity, and mortality.
DETAILED CLINICAL OBSERVATIONS: No
BODY WEIGHT: Yes
Body weights of each rat were measured at the beginning of exposure and once a week during the exposure period.
FOOD CONSUMPTION: Yes
Food consumption was measured at the beginning of exposure and once a week during the exposure period. The amounts of food were calculated before they were supplied to each cage, and their remnants were measured on the next day in order to calculate the difference, which was regarded as daily food consumption (g/rat/day).
FOOD EFFICIENCY: No data
WATER CONSUMPTION: No data
OPHTHALMOSCOPIC EXAMINATION: Yes
External eye examination on all males and females was carried out shortly before the beginning of the experiments and in the last week of the exposure period. The ocular fundus was examined in the last week of the exposure period using an indirect binocular ophthalmoscope (IO-H, Neitz Instruments Co., Japan). The conjunctiva, sclera, cornea, lens, and iris of each eye were also examined.
HAEMATOLOGY: Yes
- Time schedule for collection of blood: before necropsy
- Anaesthetic used for blood collection: Yes (sodium pentobarbital) - Animals fasted: Yes, over night
- How many animals: all
- Parameters checked: red blood cell (RBC, erythrocyte) count, hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC distribution width (RDW), platelet count, mean platelet volume (MPV), white blood cell (WBC, leukocyte) count, and differential WBC count.

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: before necropsy
- Animals fasted: Yes
- How many animals: all
- Parameters checked: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP),

creatine phosphokinase (CPK), glucose, total protein (TP), albumin, albumin/globulin ratio (A/G ratio), blood urea nitrogen (BUN), creatinine, triglyceride, phospholipid, total cholesterol, total bilirubin, calcium, and inorganic phosphorus.
URINALYSIS: Yes
During the last week of exposure, urinalysis of five males and five females per group was carried out with fresh urine to determine the specific gravity, pH, protein, glucose, ketone body, occult blood, bilirubin, urobilinogen, and nitrite by using a CliniTek-100 urine chemistry analyzer (Ames Division, Miles Laboratory, USA).
NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes
At the end of experiments, all surviving animals were anesthetized by an intraperitoneal administration of sodium pentobarbital (50 mg/kg body weight) for blood sample collection. The rats were then sacrificed by exsanguination from the abdominal aorta. Complete gross postmortem examinations were performed on all terminated animals.
ORGAN WEIGHTS
The absolute and relative (organ-to-body weight ratios) weights of the following organs were measured: brain, pituitary gland, adrenal glands, liver, spleen, kidneys, heart, lung, thymus, testes, epididymides, and ovaries.
HISTOPATHOLOGY: Yes
The following tissues were obtained from all animals: abnormal lesions, skin, mammary gland, spleen, pancreas, jejunum, stomach, duodenum, ileum, cecum, colon, mesenteric lymph node, salivary gland, submandibular lymph node, ovaries, uterus, vagina, urinary bladder, epididymides, prostates, seminal vesicles, rectum, kidneys, adrenal glands, liver, sternum, thymus, heart, lung, trachea, esophagus, thyroids (including parathyroids), tongue, aorta, sciatic nerve, skeletal muscle, femur, thoracic spinal cord, Harderian glands, brain, pituitary gland, eyes, testes, nasal cavity, nasal turbinates, and Zymbal glands. Eyes and testes were preserved in Davidson's fixative and Bouin's fixative, respectively. The other tissues were fixed with a 10% neutral buffered formalin solution. The tissues were routinely processed, embedded in paraffin, and sectioned at 3–5 µm. The sections were stained with hematoxylin–eosin stain for microscopic examination. The nasal passages and nasal turbinates were decalcified prior to being embedded and sectioned.The nasal cavity was sectioned at the levels of posterior to the upper incisors, the incisive papilla, the second palatine ridge, and the first molar teeth (Young, 1981). All organs and tissues taken from all animals in the vehicle control and the high dose groups were examined microscopically. All gross lesions, as defined by the study pathologist, were also included in the examination.

Statistics

Statistical analyses were performed by comparing the treatment groups with the vehicle control group using SAS software (SAS Institute, Inc., 1997). Data were presented as means±SD. Variance of numerical datawas checked by Bartlett's test (1937). If the variance was homogeneous, the data were subjected to oneway analysis of variance (ANOVA); if not, they were analyzed by the Kruskal–Wallis nonparametric ANOVA (1952). If either of the tests showed a significant difference among the groups, the data were analyzed by the multiple-comparison procedure of Dunnett's post hoc test (1964). Results of urinalysis obtained with reagent strips were analyzed by the Kruskal–Wallis test followed by multiple comparisons using Dunnett's test. Clinical signs, necropsy findings, and histopathological findings were represented as frequencies and were subjected to Fisher's exact probability test (1970) when necessary. The significant probability values $p < .05$ or $p < .01$ were noted.

<u>Results and discussions</u>

Effect levels

Endpoint	**Effect level**	**Based on**	**Sex**	**Basis for effect level / Remarks**
NOAEC (Systemic toxicity)	5 ppm (analytical)		male	
LOAEC (Systemic toxicity)	25 ppm (analytical)		male	Decreased body weight gain
NOAEC (Systemic toxicity)	25 ppm (analytical)		female	
LOAEC (Systemic toxicity)	125 ppm (analytical)		female	Decreased body weight gain

Results of examinations

Clinical signs and mortality : no effects

Body weight and weight gain : yes

Food consumption : yes

Food efficiency : no data

Water consumption : not examined

Ophthalmoscopic examination : no effects

Haematology : yes

Clinical chemistry : yes

Urinalysis : yes

Neurobehaviour : not examined

Organ weights : no effects

Gross pathology : no effects

Histopathology: non-neoplastic : yes

Histopathology: neoplastic : no effects

Details on results

CLINICAL SIGNS AND MORTALITY
There were no treatment-related toxic symptoms or and mortality in any of the animals treated with DMDS during the study period (data not shown).
BODY WEIGHT AND WEIGHT GAIN (Figures 1 & 2, see attached document)
The body weight gain of male rats (figure 1) was statistically significantly suppressed in the 25-ppm group on days 14, 28, 48, 56, 63, 70, 77, and 86 when compared with the control group. The body weight gain of the male 125-ppm group was also statistically significantly suppressed from day 14 on test to termination in comparison with controls. That of female rats (figure 2) was also significantly lower in the 125-ppm group on days 14 to 48 than that in the control group.
FOOD CONSUMPTION
In males, food consumption was significantly less on days 0, 7, and 63 in the 25-ppm group and on days 0, 7, and 86 in the 125-ppm group than in the vehicle control group, respectively (data not shown). In females, food consumption of the 125-ppm group was significantly decreased on days 0, 7, and 28 when compared with the vehicle control group.
FOOD EFFICIENCY
No data
WATER CONSUMPTION
Not measured
OPHTHALMOSCOPIC EXAMINATION
Ophthalmologic examinations did not show any treatment related ocular lesions in any of the animals (data not shown).
HAEMATOLOGY (Table 1)
RBC and hematocrit were statistically significantly increased in the male 5-ppm group when compared with the control group. RBC, WBC, and lymphocytes were also statistically significantly increased in the male 25-ppm group, and MCVand MCH were significantly decreased in comparison with those of the control group. The male 125-ppm group showed a statistically significant increase in the RBC, and a statistically significant decrease in the MCV and MCH compared with the controls. In females (data not shown), a statistically significant decrease in the hemoglobin concentration was found in the 125-ppm group when compared with the control group.However, all these variations are not considered to be treatment-related because there are of low intensity and/or not dose-related and in the range of the historical control data.
CLINICAL CHEMISTRY (Tables 2 and 3)
In males, a statistically significant decrease in AST, ALT, and BUN was observed in the 25-ppm group in comparison with the control group. The male 125-ppm group showed a statistically significant decrease in AST, ALT, BUN, CPK, and triglyceride, and a statistically significant increase in serum glucose when compared with the control group. In females, a statistically significant decrease in AST and BUN was observed in the 25-ppm group when compared with the control group. The 125-ppm group exhibited a statistically significant decrease in AST, BUN, and CPK when compared with the control group.However, all these variations are not considered to be treatment-related because there are of low intensity and/or not dose-related and in the range of the historical control data.
URINALYSIS
A significant decrease in the specific gravity and white blood cell at 5 ppm and the white blood cell at 125 ppm was observed in males without any dose-response relationship (data not shown). There were no significant differences between the treatment groups and controls regarding any of the urinary parameters examined in females.
NEUROBEHAVIOUR
Not examinated

ORGAN WEIGHTS (Tables 4 and 5)
In males, absolute liver weight was significantly decreased in the 125-ppm group and relative weights of kidneys and adrenal glands were significantly increased in the same dose group in comparison with the control group. In females, absolute thymus weight in the 125-ppm group was significantly lower than that in the control group, while there were no significant differences in the relative organ weights between the control and treatment groups.
GROSS PATHOLOGY
At the scheduled necropsy, a dark red spot on the thymus was observed in a single male of the control group, but there were no treatment-related gross findings in any of the treated animals (data not shown).
HISTOPATHOLOGY: NON-NEOPLASTIC
In males (data not shown), bile duct proliferation, macrophage infiltration in the bronchioles, atrophy of the salivary glands, pseudogland formation in the nasal cavity, atrophy of the prostate, and chromodacryorrhea of the Harderian gland were observed in 1 case each in the 125-ppm group. Two cases of a goblet cell proliferation in the nasal cavity, 3 cases of glomerular atrophy of the kidney, 5 cases of protein cast in the kidney, 6 cases of epithelial destruction of the small intestinal villi, 8 cases of functional hyperplasia of the prostate, and 10 cases of bile pigment infiltration in the spleen were also observed in the 125-ppm group. In females, centriacinar telangiectasis, bile-duct proliferation, protein cast in the kidney, ultimobranchial cyst in the thyroid gland, ectopic thymus, and lymphoid cell infiltration in the Harderian gland were observed in 1 case each in the 125-ppm group. Two cases of chromodacryorrhea of the Harderian gland, 3 cases each of epithelial destruction of the small intestinal villi and goblet-cell proliferation in the nasal cavity, and 10 cases of bile pigment infiltration in the spleen were also observed in the 125-ppm group. The incidence and severity of the histopathological alterations observed in the high dose group of both genders were similar to controls.

Any other information on results incl. tables

TABLE 1 : Haematological findings in male rats exposed to dimethyl disulfide for 13 wk

	Dimethyl disulfide (ppm)			
Parameters	0	5	25	125
RBC (×1012/L)	8.47±0.575	9.34±0.586** #	9.30±0.455** #	9.25±0.484* #
HB (g/dl)	15.65±0.433	16.19±0.943	16.01±0.500	15.45±0.493
HCT (%)	39.73±2.653	42.97±2.573*#	42.34±1.819	41.72±2.270
MCV (fl)	46.91±0.631	46.03±0.996	45.54±0.648** #	45.12±0.658** #
MCH (pg)	18.56±1.301	17.37±0.929	17.26±1.121* #	16.74±0.847** #
MCHC (g/dl)	39.53±2.673	37.74±2.196	37.89±2.175	37.12±1.909
RDW (%)	15.17±0.254	15.21±0.376	15.13±0.250	15.42±0.336
PLT (×109/L)	522.5±21.83	559.3±58.13	582.0±156.9	584.9±29.30
WBC (×109/L)	4.17±0.578	4.78±0.890	5.41±0.597**#	4.60±0.534
NEU (×109/L)	1.03±0.217	1.40±0.319	1.20±0.246	1.07±0.200
LYM (×109/L)	2.95±0.436	3.19±0.786	4.05±0.473**	3.36±0.465
MON (×109/L)	0.18±0.051	0.19±0.076	0.15±0.040	0.17±0.047
EOS (×109/L)	0.002±0.004	0.005±0.007	0.009±0.009	0.001±0.003
BAS (×109/L)	0.00±0.000	0.001±0.003	0.004±0.007	0.000±0.000

*Note.*Values are presented as mean±SD. RBC, red blood cells; HB, hemoglobin, HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; PLT, platelet; NEU, neutrophil; LYM, lymphocyte; MON, monocyte; EOS, eosinophil; and BAS, basophil.
*Significant difference at*p*.05 level when compared with the control group.
**Significant difference at*p*.01 level when compared with the control group.
values in the range of the laboratory's historical data (n=84) (Kang BH, Son HY, Ha CS et al. (1995) Reference values of hematology and serum chemistry in Ktc: Sprague-Dawley rats. Korean J Lab Anim Sci; 11: 141-5): RBC: 7.41 - 9.96, HCT: 38.17 - 53.28, MCV: 49.30 - 56.39, MCH: 16.35 - 19.83, WBC: 4.68 - 17.85.

TABLE 2 : Serum biochemical findings in male rats exposed to dimethyl disulfide for 13 wk

	Dimethyl disulfide (ppm)			
Parameters	0	5	25	125
AST (IU/L)	169.2±21.61	143.8±28.77	122.6±26.35** #	110.7±10.58** #
ALT (IU/dl)	68.6±17.91	59.9±11.84	45.8±7.75**#	47.9±5.17** #
ALP (mg/dl)	510±49.2	508±54.6	513±59.5	480±56.7
BUN (mg/dl)	18.6±1.50	18.2±1.16	16.9±1.28*#	15.9±1.61** #
CRTN (mg/dl)	0.59±0.102	0.52±0.096	0.48±0.083* #	0.54±0.050

GLU (mg/dl)	128.5±17.38	163.5±54.59	154.1±35.60	182.5±23.14** #
T-CHO (mg/dl)	68.8±5.96	74.1±5.88	65.3±6.20	66.0±6.13
T-BIL (mg/dl)	0.101±0.008	0.095±0.016	0.101±0.010	0.108±0.008
TP (g/dl)	6.59±0.199	6.77±0.391	6.38±0.246	6.50±0.164
ALB (g/dl)	4.42±0.075	4.53±0.145	4.43±0.115	4.39±0.152
CPK (IU/L)	1630±626.0 ##	1268±442.1 ##	1187±361.7 ##	1038±398.3* ##
TG (mg/dl)	75.5±34.19	64.8±24.30	75.1±27.29	37.9±19.70** #
Ca (mg/dl)	9.30±0.121	9.69±0.838	9.22±0.239	9.16±0.186
IP (mg/dl)	5.96±1.283	7.66±2.889	6.05±0.586	5.93±0.551
PL (mg/dl)	120.3±8.14	122.3±9.59	114.7±10.29	117.1±6.01
A/G (ratio)	2.04±0.135	2.04±0.162	2.28±0.169	2.10±0.258

*Note.*Values are presented as means±SD. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; CRTN, creatinine; GLU, glucose; T-CHO, total cholesterol; T-BIL, total bilirubin; TP, total protein; ALB, albumin; CPK, creatine phosphokinase; TG, triglyceride; Ca, calcium; IP, inorganic phosphate; PL, phospholipid; and A/G, albumin/globulin.
*Significant difference at*p*.05 level when compared with the control group.
**Significant difference at*p*.01 level when compared with the control group.
values in the range of the laboratory's historical data (n=84) (Kang BH, Son HY, Ha CS et al. (1995) Reference values of hematology and serum chemistry in Ktc: Sprague-Dawley rats. Korean J Lab Anim Sci; 11: 141-5): AST : 50.46 - 171.97, ALT: 25.29 - 84.24, BUN: 11.64 - 20.20, CRTN: 0.19 - 0.85, GLU: 104.25 - 217.56, TG : 12.20 - 242.35
value higher than the historical control data in all groups (CPK: 67.80 - 620.20)

TABLE 3 : Serum biochemical findings in female rats exposed to dimethyl disulfide for 13 wk

	Dimethyl disulfide (ppm)			
Parameters	0	5	25	125
AST (IU/L)	122.1±20.40	111.2±12.20	98.0±16.07** #	96.2±8.27** #
ALT (IU/dl)	43.7±8.45	45.9±8.22	41.7±8.91	39.6±5.38
ALP (mg/dl)	488±86.8	472±38.2	457±57.8	444±58.1
BUN (mg/dl)	18.6±2.31	17.9±1.97	16.0±1.70* #	15.0±1.39** #
CRTN (mg/dl)	0.45±0.084	0.44±0.050	0.47±0.056	0.46±0.062
GLU (mg/dl)	97.6±22.74	117.3±24.82	116.4±20.38	121.8±27.29
T-CHO (mg/dl)	82.8±12.26	84.3±11.58	78.5±7.95	81.0±8.52
T-BIL (mg/dl)	0.105±0.008	0.104±0.008	0.101±0.013	0.121±0.008
TP (g/dl)	6.21±0.378	6.09±0.316	6.27±0.304	6.30±0.158
ALB (g/dl)	4.27±0.133	4.29±0.108	4.32±0.100	4.29±0.061
CPK (IU/L)	970±298.3 ##	725±162.8 ##	820±521.3 ##	617±208.7* ##
TG (mg/dl)	11.8±3.34	11.7±6.82	10.7±1.49	10.8±1.96
Ca (mg/dl)	7.08±1.660	6.08±0.639	6.62±1.441	7.18±1.314
IP (mg/dl)	5.96±1.283	7.66±2.889	6.05±0.586	5.93±0.551
PL (mg/dl)	136.6±20.05	142.5±19.23	133.8±11.90	141.5±14.52
A/G (ratio)	2.24±0.342	2.42±0.360	2.25±0.251	2.15±0.155

*Note.*Values are presented as means±SD. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; CRTN, creatinine; GLU, glucose; T-CHO, total cholesterol; T-BIL, total bilirubin; TP, total protein; ALB, albumin; CPK, creatine phosphokinase; TG, triglyceride; Ca, calcium; IP, inorganic phosphate; PL, phospholipid; and A/G, albumin/globulin.
* Significant difference at*p*.05 level when compared with the control group.
** Significant difference at*p*.01 level when compared with the control group.
# values in the range of the laboratory's historical data (n=84) (Kang BH, Son HY, Ha CS et al. (1995) Reference values of hematology and serum chemistry in Ktc: Sprague-Dawley rats. Korean J Lab Anim Sci; 11: 141-5): AST : 58.97 - 173.75, BUN : 11.58 - 25.60
value higher than the historical control data in all groups (CPK: 68.70 - 476.56)

<u>Overall remarks, attachments</u>

Remarks on results including tables and figures

TABLE 4: Absolute and relative organ weights in male rats exposed to dimethyl disulfide for 13 wk

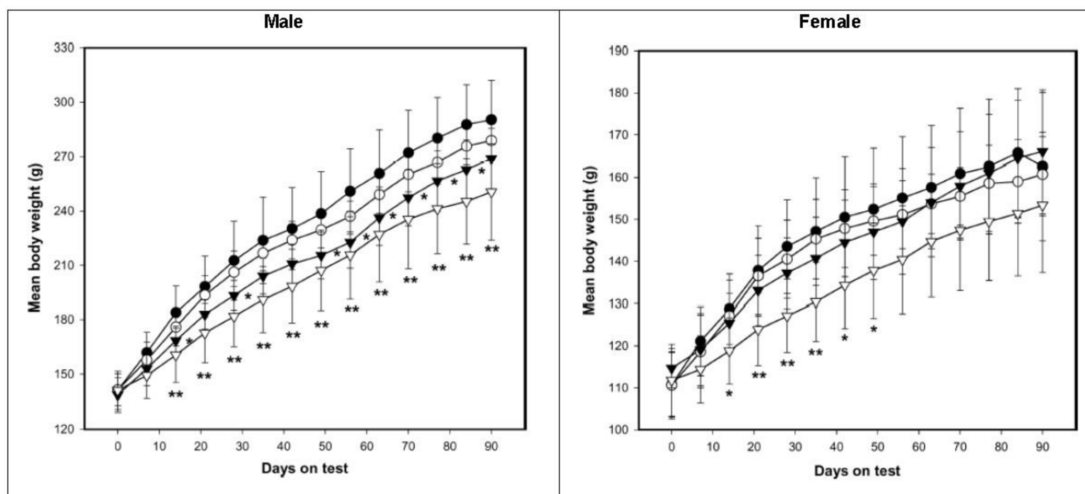
	Dimethyl disulfide (ppm)			
Parameters	0	5	25	125
Thymus	0.239±0.089	0.226±0.031	0.227±0.072	0.229±0.091
per body weight (%)	0.088±0.029	0.087±0.013	0.090±0.022	0.099±0.036
Heart	0.891±0.155	0.835±0.111	0.811±0.106	0.761±0.107
per body weight (%)	0.331±0.059	0.322±0.038	0.325±0.038	0.328±0.038
Adrenal glands	0.037±0.020	0.038±0.011	0.044±0.020	0.054±0.016
per body weight (%)	0.014±0.008	0.015±0.005	0.018±0.008	0.023±0.006*
Testes	2.975±0.764	2.700±0.185	2.754±0.100	2.968±0.209
per body weight (%)	1.099±0.245	1.043±0.044	1.108±0.075	1.168±0.074
Epididymides	0.907±0.122	0.905±0.098	0.846±0.105	0.833±0.129
per body weight (%)	0.337±0.045	0.350±0.041	0.338±0.027	0.360±0.043
Lung	1.004±0.190	1.021±0.054	0.974±0.087	0.915±0.150
per body weight (%)	0.370±0.046	0.395±0.018	0.391±0.025	0.393±0.033
Kidneys	1.720±1.585	1.729±0.098	1.682±0.143	1.719±0.202
per body weight (%)	0.637±0.038	0.669±0.047	0.674±0.033	0.741±0.037**
Spleen	0.513±0.101	0.530±0.075	0.492±0.043	0.449±0.062
per body weight (%)	0.189±0.027	0.204±0.021	0.198±0.019	0.193±0.012
Liver	7.843±0.615	7.545±0.040	7.370±0.743	6.947±0.836*
per body weight (%)	2.905±0.115	2.918±0.169	2.951±0.142	2.990±0.089
Brain	1.812±0.292	1.730±0.1312	1.770±0.123	1.697±0.140
per body weight (%)	0.674±0.123	0.669±0.050	0.712±0.066	0.737±0.086
Pituitary gland	0.005±0.003	0.006±0.004	0.005±0.004	0.004±0.002
per body weight (%)	0.002±0.001	0.002±0.002	0.002±0.001	0.002±0.001

TABLE 5: Absolute and relative organ weights in female rats exposed to dimethyl disulfide for 13 wk

	Dimethyl disulfide (ppm)			
Parameters	0	5	25	125
Thymus	0.218±0.067	0.179±0.049	0.197±0.041	0.156±0.025*
per body weight (%)	0.146±0.036	0.121±0.028	0.129±0.028	0.112±0.032
Heart	0.512±0.074	0.498±0.038	0.501±0.064	0.500±0.060
per body weight (%)	0.346±0.059	0.338±0.036	0.327±0.019	0.352±0.021
Adrenal glands	0.048±0.012	0.046±0.012	0.050±0.004	0.051±0.012
per body weight (%)	0.032±0.008	0.031±0.008	0.033±0.004	0.036±0.007
Ovaries	0.079±0.086	0.083±0.059	0.101±0.073	0.075±0.037
per body weight (%)	0.055±0.065	0.057±0.042	0.067±0.054	0.053±0.027
Lung	0.739±0.097	0.718±0.070	0.713±0.078	0.698±0.079
per body weight (%)	0.494±0.025	0.486±0.029	0.468±0.062	0.493±0.056
Kidneys	1.085±0.073	1.063±0.075	1.102±0.101	1.077±0.101
per body weight (%)	0.732±0.050	0.720±0.037	0.720±0.026	0.760±0.041
Spleen	0.369±0.025	0.343±0.032	0.346±0.059	0.320±0.046
per body weight (%)	0.251±0.038	0.232±0.017	0.226±0.032	0.224±0.015
Liver	3.904±0.518	3.875±0.371	3.965±0.366	3.768±0.517
per body weight (%)	2.616±0.171	2.622±0.192	2.593±0.152	2.644±0.162
Brain	1.649±0.102	1.672±0.061	1.603±0.127	1.561±0.151
per body weight (%)	1.117±0.128	1.136±0.878	1.050±0.066	1.110±0.157
Pituitary gland	0.005±0.003	0.004±0.002	0.005±0.002	0.003±0.002
per body weight (%)	0.003±0.002	0.002±0.001	0.003±0.002	0.002±0.001

Note. Values are presented as mean±SD (g).
* Significant difference at*p*.05 level when compared with the control group.
** Significant difference at*p*.01 level when compared with the control group.

Mean body weights for male and female rats exposed to dimethyl disulphide at concentrations of 0 (●), 5 (○), 25 (▼), and 125 (▽) ppm. Values are presented as means ± SD. Asterisk and double asterisk indicate significant difference at p < .05 and p < .01 levels, respectively, when compared with the control group.



__Applicant's summary and conclusion__

Conclusions

The 13-wk repeated exposure of rats to DMDS caused decreases in the body weight gain and food intake in males at 25 ppm and decreases in the body weight gain, food intake and thymus weight and increases adrenal glands weight at 125 ppm of both sexes. The target organ could not be determined in rats under these experimental conditions. The no-observed-adverse-effect concentration (NOAEC) was considered to be 5 ppm, 6 h/day for male rats and 25 ppm, 6 day for female rats, respectively.

Executive summary

In a study was carried out following the OECD guideline # 413, groups of 10 F344 rats of each sex were exposed to dimethyl disulphide (DMDS) vapor by whole-body exposure at concentrations of 0, 5, 25, or 125 ppm for 6 h/day, 5 days/wk for 13 wk. All the rats were sacrificed at the end of treatment period. During the test period, clinical signs, mortality, body weights, food consumption, ophthalmoscopy, urinalysis, hematology, serum biochemistry, gross findings, organ weights, and histopathology were examined. At 25 ppm, a decrease in the body weight gain and food intake was observed in the males, but not in the females. However, at 125 ppm, a decrease in the body weight gain, food intake, and thymus weight and an increase in the weights of adrenal glands were observed in both genders. In contrast, no treatment-related effects were observed in the 5 ppm group. In these experimental conditions, the target organ was not determined in rats. The no-observed-adverse-effect concentration (NOAEC) was found to be 5 ppm, 6 h/day for male rats and 25 ppm, 6 h/day for female rats.

[Study 3] Nemec (WIL) 2006/K1 KS/90-day inhalation (neurotoxicity)toxicity, rat

__Study Reference__

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	Key Stduy	1 (reliable without restriction)	Nemec MD	2006	A subchronic inhalation neurotoxicity study of dimethyl disulfide in rats

__Materials and methods__

Test type : subchronic

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD Guideline 424 (Neurotoxicity Study in Rodents)	no

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : male/female

Administration / exposure

Route of administration : inhalation: vapour

Type of inhalation exposure : whole body

Vehicle : clean air

Analytical verification of doses or concentrations : yes

Duration of treatment / exposure : 13 weeks

Frequency of treatment : 6 hours per day, 7 days per week

Doses/concentrations : 5, 20 and 80 ppm

Basis analytical conc.

No. of animals per sex per dose : 12

Control animals : yes, sham-exposed

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
NOAEC (systemic toxicity and neurotoxicity)	20 ppm (analytical)		male	Lower body weight gains, lower food consumption and lower total session motor activity counts at 80 ppm.
NOAEC (systemic toxicity and neurotoxicity)	>= 80 ppm (analytical)		female	No adverse effect at 80 ppm.
NOAEC (local contact (nasal) irritation)	5 ppm (analytical)		male/female	Mild to moderate degeneration of the nasal olfactory epithelium at 20 and 80 ppm.

Applicant's summary and conclusion

Conclusions

Systemic toxicity of DMDS administered by whole-body inhalation exposure to Crl:CD(SD) rats for 13 weeks was observed at an exposure level of 80 ppm in males as evidenced by lower body weight gains and lower food consumption through the first 8 weeks of the exposure period. Lower total session motor activity counts were noted in

the 80 ppm group males during study week 12. Local contact (nasal) irritation was observed at exposure levels of 20 and 80 ppm in males and females as a dose-dependent mild to moderate degeneration of the nasal olfactory epithelium.

Executive summary

A subchronic neurotoxicity study via the inhalation route was conducted with dimethyl disulphide (DMDS) following the OECD guideline # 424. Four groups of 12 male and 12 female Crl:CD(SD)BR were exposed to either clean filtered or DMDS vapor atmospheres or 5, 20 or 80 ppm for 6 hours daily in whole-body inhalation chambers for 13 consecutive weeks. All animals were observed twice daily for mortality and moribundity and clinical examinations were perfortned daily. Body weights and food consumption were recorded weekly. Functional observational battery and locomotor activity assessments were evaluated prior to initiation of exposure and at the time of peak effect (one hour post exposure) during study weeks 3, 7 and 12. Brain weights and dimensions were determined and neuropathologic evaluations were performed. In addition, a microscopic examination of nasal tissues was performed.
DMDS-related, dose-dependent findings in the 20 and 80 ppm group males and females included lower food consumption and corresponding lower body weight gains during study week 0 to 1. Thereafter, body weight gain and food consumption for the 20 and 80 ppm group females and 20 ppm group males returned to levels comparable to the control group beginning during study week 1 to 2, but lower food consumption and body weight gains were sustained in the 80 ppm group males for the duration of the study. As a result, body weights of the males were reduced compared to the control group and cumulative body weight gains were reduced for the duration of the study for both sexes.
Lower total motor activity counts were observed in the 80 ppm group males during all sub-intervals of the study week 12 evaluation. Habituation patterns for these animals were unremarkable. Neuropathologic parameters affected by test article exposure included lower brain length in the males. Minimal to moderate degeneration of the olfactory epithelium on nasal Level II was observed in all 80 ppm males and females. In general, the olfactory epithelium of the females was more severely affected. Minimal to moderate degeneration of the olfactory epithelium was noted on nasal Level II in 6/6 males and 4/6 females in the 20 ppm group. Olfactory epithelial degeneration, characterized by a loss of the adluminal cytoplasmic layer of the sustentacular cells, was also found on Levels III and IV in the 80 ppm group. Minimal olfactory epithelial degeneration on Levels III and IV was noted in 1/6 males in the 20 ppm group. The only test article-related finding in the 5 ppm group consisted of minimal degeneration of the olfactory nasal epithelium on nasal Level III in 1/6 males. On Level II, degeneration of the olfactory epithelium was most noticeable on the dorsal arches, while on Levels III and IV, the alteration typically affected the medial aspects of turbinates in the dorsal meatus.
The no-observed-adverse-effect concentration (NOAEC) for systemic toxicity and neurotoxicity of DMDS via whole-body inhalation exposure to Crl:CD(SD) rats for 13 consecutive weeks was 20 ppm for males and 80 ppm for females. The NOAEL for local contact (nasal) irritation of DMDS via whole-body inhalation exposure for 13 consecutive weeks was 5 ppm for both males and females.

3.9 Repeated dose toxicity – dermal route

[Study 1] Prinsen (CIVO-TNO) 1990/K1 KS/28-d dermal toxicity

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key Stduy	1 (reliable without restriction)	Prinsen MK	1990	Repeated-dose (28-day) dermal toxicity study with|Dimethyl Disulfide (DMDS) in rabbits

Materials and methods

Test type : subacute

Limit test : no

Test guideline

Qualifier	Guideline	Deviations

according to	OECD Guideline 410 (Repeated Dose Dermal Toxicity: 21/28-Day Study)	

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.88%

Test animals

Species : rabbit

Strain : New Zealand White

Sex : male/female

Details on test animals and environmental conditions
TEST ORGANISMS:
- Source: ENKI-Konijnenfarm, someren, the Netherlands
- Age at reception: 12 weeks old - Weight at the start of the treatment: no data
- Number of animals: The control and top-dose group comprised 10 males and 10 females, whereas the low- and mid-dose group comprised 5 males and 5 females.
- Aclimatation period: 13 days

HOUSING The animals were housed individually in suspended, galvanized cages, fitted with a wire-mesh floor and front.

FOOD and WATER
- Food: standard laboratory rabbit diet ad libitum
- Water: tap water, ad libitum

ENVIRONMENTAL CONDITIONS
- Temperature : 18 ± 3°C
- Relative humidity : at least 40%
- Light/dark cycle : 12h/12h
- Ventilation : at least 10 changes/hour

Administration / exposure

Type of coverage : occlusive

Vehicle : unchanged (no vehicle)

Details on exposure
Doses were applied by volume, viz. 1.0, 0.1, and 0.01 ml/kg body weight for the top-dose, the mid-dose, and the low-dose, respectively. The respective amounts of the test substance were applied topically to the intact, shaven skin area by means of a syringe fitted with a blunt needle. Upon contact with the shaven skin, the test substance immediately spread spontaneously, so covering application sites of circa 2 x 2 cm, 6 x 6 cm, and 15 x 15 cm in the low-, mid-, and top-dose group, respectively. Immediately after application, the test site of each rabbit was covered with porous gauze dressing fixed onto a non-irritating tape. The entire trunk of each rabbit was wrapped with this tape to maintain the gauze dressing in position and to retard evaporation of volatile substances. The animals of the control group were sham-treated with the patches only. The dermal exposure period was approximately 6 hours per day and 5 days per week for a period of 4 weeks for the control, low-dose, and mid-dose group. Because of the high mortality that occurred in the top-dose group in week 3 of the study, the animals of this group were treated only during the first 2 1/2 week of the study, i.e. 13 exposures to DMDS.

Details on analytical verification of doses or concentrations : Not appropriate

Duration of treatment / exposure

0.01 and 0.1 ml/kg/d groups were treated 5 days/week during a four-week period, whereas the 1 ml/kg/d group was treated with for 2 1/2 weeks (i.e. 13 days of treatment).

Frequency of treatment

DMDS was administered daily, by dermal occlusive application (6 hours daily) to four groups of albino rabbits.

Doses/concentrations : 0.01, 0.1, 1 ml/kg/day (10.63, 106.3 and 1063 mg/kg bw/d)

Basis nominal per unit body weight

No. of animals per sex per dose : The control and 1.0 ml/kg/d group consisting of 10 males and 10 females, and the 0.01 and 0.1 ml/kg/d group consisting of 5 males and 5 females.

Control animals : other: sham treated with the occlusive dressing

Details on study design

After nominal day 16 of the study, the surviving animals of the top-dose group were kept without further treatment during the remainder of the study.

Positive control

Not appropriate

Examinations

Observations and examinations performed and frequency

- Clinical signs: twice a day on exposure days and once a day on non-exposure days.
- Mortality: twice a day.
- Dermal reactions: At the start of the study and prior to each daily administration, individual skin reactions were evaluated in all (four) groups by the method of Draize et al. (J. Pharmacol. Exp. Ther. 82 (1944) 377-390). In addition, on day 28 and day 29 of the study just prior to autopsy, again skin effects were recorded.
- Body weight: at the start of the study, twice a week thereafter, and on the day of autopsy, i.e. on day 0, 3, 7, 10, 14, 17, 21, 24, 28, and day 29 of the study.
- Food consumption: on day 0-3, day 3-7, day 7-10, day 10-14, day 14-17, day 17-21, day 21-24, and day 24-28. - Ophthalmoscopic examination: no
- Blood examinatrions: On nominal day 23 for males and on nominal day 24 for females (Le. 7 and 8 days after the last treatment to DMDS for males and females of the top-dose group, respectively), haematology and clinical chemistry determinations were conducted in blood or plasma of the animals, which were deprived of food for approximately 24 hours prior to the time of blood sampling

* Haematology: Hemoglobin, hematocrit, red blood cell count, white blood cell count, differential leukocyte count, platelet count, mean cell volume, mean cell haemoglobin concentration, mean cell haemoglobin

* Biochemistry:

. Electrolytes: calcium, chloride, phosphorous, potassium, sodium,

. Enzymes: alkaline phosphatase, alanine-aminotransferase, aspartate-aminotransferase, gamma-glutamyl-transferase

. Other: albumin, blood creatinine, blood urea nitrogen, albumin/globulin, glucose, total bilirubin, total cholesterol, total serum protein, bile acids

- Urinanalysis: no

Sacrifice and pathology

On day 28 and 29 of the study (i.e. 12 and 13 days after the last treatment to DMDS for males and females of the top-dose group), animals were sedated by intravenous injection with Nembutaltm and subsequently killed by opening the abdominal aorta. Next, the animals were examined grossly for pathological changes. - Weighted organs: adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes, thyroid and thymus. - Microscopic examinations: Histopathological examination was done on the skin (treated and untreated), heart, brain, lungs, trachea, liver, kidneys, adrenals, spleen, testes, ovaries and thymus of the animals of the control group, the mid-dose and the high-dose groups. Examination of the bone-marrow (sternum), popliteal, submandibular and mesenteric lymph nodes, spinal corde and ciatic nerve were carried out in the rabbits of the control- and the top-dose group. The skin, the thymus and the heart were also examined in the low- dose group because of possible treatment-related effects. In addition, histopathological examination was also done on the brain of the animals of the control group, the mid-dose, and the top-dose group.

Statistics

Body-weight: Covar + Dunnetts tests (two-sided)Food intake: Anova + Dunnetts tests (two-sided)Haematology: Anova + Dunnetts tests (two-sided) and Kruskal-Wallis Anova + Mann Whitney u-testBlood chemistry: Anova + Dunnetts tests (two-sided)Organ weight: Anova + Dunnetts tests (two-sided)Histophatology: pairwise Fisher's test

Results and discussions

Effect levels

Endpoint	**Effect level**	**Based on**	**Sex**	**Basis for effect level / Remarks**
NOEL (systemic toxicity)	10.63 mg/kg bw/day		male/female	
NOAEL (systemic toxicity)	106 mg/kg bw/day		male/female	A slight and reversible lethargy after each administration
LOAEL (local irritation)	8.5 mg/cm² per day (nominal)		male/female	skin irritation observed at all dose levels

Results of examinations

Clinical signs and mortality : yes

Dermal irritation : yes

Body weight and weight gain : yes

Food consumption : yes

Food efficiency : no data

Water consumption : not examined

Ophthalmoscopic examination : not examined

Haematology : yes

Clinical chemistry : yes

Urinalysis : not examined

Neurobehaviour : yes

Organ weights : yes

Gross pathology : yes

Histopathology: non-neoplastic : yes

Histopathology: neoplastic : not examined

Details on results

CLINICAL SIGNS: After each daily treatment with DMDS, temporary effects on the central nervous system (CNS) were observed in the animals of the 0.1 and 1 ml/kg/d group. The observed behavioural effects consisted of slight lethargy in the 0.1 ml/kg/d group and distinct to severe lethargy and unconscinousness in the 1 ml/kg/d group. At the end of each daily exposure these effects were no longer observed. During the four-week test period, treatment-related signs of abnormal behaviour were not observed in the animals of the 0.01 ml/kg/d group or in the controls.
MORTALITY: During the second and third week of the study treatment-related mortality occurred in males and females of the 1 ml/kg/d group. Therefore, it was decided to discontinue the treatment of the 1 ml/kg/d group on nominal day 16 of the study, i.e. after 13 days of treatment.
SKIN REACTIONS: Repeated dermal administration of DMDS caused severe, dose-dependent skin irritation in all dose groups. During the treatment period, decreased body weights were observed in males of the 1 ml/kg/d group.
FOOD CONSUMPTION and BODY WEIGHT: Food intake of males was somewhat decreased during the same period in the 1 ml/kg/d group. Mean body weight and food intake figures of the 0.01 and 0.1 ml/kg/d group were comparable with those of the controls.
BLOOD EXAMINATIONS: Haematology and clinical chemistry examinations of the 1 ml/kg/d males, 7 days after the last exposure to DMDS, revealed treatment-related changes in several red blood cell variables and in the number of

white blood cells, and treatment-related changes in plasma creatinine and sodium concentrations. In the females no effects on clinical chemistry variables were observed that could be ascribed to treatment with DMDS.
PATHOLOGY: The absolute and relative organ weights measured at autopsy did not show statistically significant differences that could be ascribed to treatment. However, the mean thymus weight of both males and females tended to be lower in the two higher dose groups than in the controls. Macroscopic examination at autopsy did not reveal any treatment-related changes other than the dermal lesions induced during the treatment with DMDS. Microscopic examination revealed treatment-related changes in the heart of some males and females of the 1 ml/kg/d group and in the treated skin of all three dose-groups, confirming the macroscopic lesions observed during the study and at autopsy. No treatment-related changes were found in the brain, spinal cord, sciatic nerve, or thymus.

Applicant's summary and conclusion

Conclusions

A dose of 1.0 ml or 1063 mg/kg bw/d applied dermally to rabbit for 13 days is a clear effect level. The NOAEL of DMDS for systemic toxicity is considered at 0.1 ml or 106.3 mg/kg bw/d based on slight transient lethargic effects. The overall NOAEL of DMDS including local skin effects is lower than 0.01 ml or 10.63 mg/kg bw/d.

Executive summary

In a study performed according to the OECD guideline # 410, dimethyl disulphide was administered daily, by dermal occlusive application (6 hours daily) to four groups of albino rabbits. The dose levels applied were 0, 0.01, 0.1, and 1.0 ml/kg body weight/day, which is equivalent to 0, 10.63, 106.3, and 1063 mg/kg body weight/day, respectively. The control and 1.0 ml/kg/d group consisting of 10 males and 10 females, and the 0.01 and 0.1 ml/kg/d group consisting of 5 males and 5 females.. The animals of the 0.01 and 0.1 ml/kg/d group were treated five days a week during a four-week period, whereas animals of the 1 ml/kg/d group were treated with DMDS for 2 1/2 weeks (i.e. 13 days of treatment).
After each daily treatment with DMDS, temporary effects on the central nervous system (CNS) were observed in the animals of the 0.1 and 1 ml/kg/d group. The observed behavioural effects consisted of slight lethargy in the 0.1 ml/kg/d group and distinct to severe lethargy and unconscinousness in the 1 ml/kg/d group. At the end of each daily exposure these effects were no longer observed. During the four-week test period, treatment-related signs of abnormal behaviour were not observed in the animals of the 0.01 ml/kg/d group or in the controls. During the second and third week of the study treatment-related mortality occurred in males and females of the 1 ml/kg/d group. Therefore, it was decided to discontinue the treatment of the 1 ml/kg/d group on nominal day 16 of the study, i.e. after 13 days of treatment.
Repeated dermal administration of DMDS caused severe, dose-dependent skin irritation in all dose groups.
During the treatment period, decreased body weights were observed in males of the 1 ml/kg/d group. Food intake of males was somewhat decreased during the same period in the 1 ml/kg/d group. Mean body weight and food intake figures of the 0.01 and 0.1 ml/kg/d group were comparable with those of the controls.
Haematology and clinical chemistry examinations of the 1 ml/kg/d males, 7 days after the last exposure to DMDS, revealed treatment-related changes in several red blood cell variables and in the number of white blood cells, and treatment-related changes in plasma creatinine and sodium concentrations. In the females no effects on clinical chemistry variables were observed that could be ascribed to treatment with DMDS.
The absolute and relative organ weights measured at autopsy did not show statistically significant differences that could be ascribed to treatment. However, the mean thymus weight of both males and females tended to be lower in the two higher dose groups than in the controls.
Macroscopic examination at autopsy did not reveal any treatment-related changes other than the dermal lesions induced during the treatment with DMDS. Microscopic examination revealed treatment-related changes in the heart of males and females of the 1 ml/kg/d group and in the treated skin of all three dose-groups, confirming the macroscopic lesions observed during the study and at autopsy. No treatment-related changes were found in the brain, spinal cord, sciatic nerve, or thymus.
The dose of 1.0 ml/kg/day (1063 mg/kg bw/day) is a clear effect level and exceeds the maximal tolareted dose. The no-adverse-effect level (NOAEL) of DMDS forsystemic toxicityis 0.1 ml/kg/day (106.3 mg/kg bw/day) and the NOAEL for local irritation is less than0.01 ml/kg/day (10.63 mg/kg bw/day).

3.10 Germ cell mutagenicity

3.10.1 In vitro data

[Study 1] Wagner VO (BioReliance) 2007/K1 KS/Ames test

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key Stduy	1 (reliable without restriction)	Wagner VO	2007	Dimethyl disulfide. Bacterial reverse mutation assay

Materials and methods

Type of genotoxicity : gene mutation

Type of study : bacterial reverse mutation assay (e.g. Ames test)

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 471 (Bacterial Reverse Mutation Assay)	no
according to	EPA OPPTS 870.5100 - Bacterial Reverse Mutation Test (August 1998)	

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.2%

Method

Target gene : Histidine locus (Salmonella) and tryptophane locus (Escherichia)

Species/strain : S. typhimurium TA 1535, TA 1537, TA 98 and TA 100

Metabolic activation: with and without

Metabolic activation system Liver S9 homogenate was prepared from rats that have been induced with Arochlor 1254

Species/strain E. coli WP2 uvr A

Metabolic activation with and without

Metabolic activation system Liver S9 homogenate was prepared from rats that have been induced with Arochlor 1254

Test concentrations

The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate in the initial toxicity-mutation assay and 50, 150, 500, 1500 and 5000 µg per plate in the confirmatory mutagenicity assay.

Vehicle : Dimethyl sulphoxide

Controls

Negative controls yes

Solvent / vehicle controls yes (DMSO)

True negative controls no

Positive controls yes

Positive control substance other: With S9: 2-aminoanthracene, 1 µg/plate for all Salmonella strains, 10µg/plate for

E. coli. Without S9: TA 98, 2-nitrofluorene (1 µg/plate); TA100 and TA 1535, sodium azide (1 µg/plate); TA 1537, 9-aminoacridine (75 µg/plate); E. coli, MMS (1000 µg/plate)

Details on test system and conditions

DETERMINATION OF CYTOTOXICITY
- Method: relative total growth (decrease in the number of revertant colonies and/or a thinning of the bacterial lawn);

EXPERIMENTS
- Initial toxicity-mutation assay: in all strains, with or without S9 mix ; 8 dose-levels (2 plates/dose level)- Confirmatory mutagenicity assay: in all strains, with or without S9 mix ; 5 dose-levels (3 plates/dose level)

METHOD OF APPLICATION: Direct plate incorporation method: for preliminary both experiments

DURATION
- Exposure duration: 48-72H

Evaluation criteria

Reproducible increase in the number of revertant colonies (2-fold for TA98/TA100 and WP2 uvrA, 3-fold for TA 1535/TA 1537) compared with vehicle controls in any strain at any dose-level and/or evidence of a dose-relationship. Reference to historical data and consideration to biological relevance may also be taken into account.

Results and discussions

Test results

Species/strain	other: S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 and E. coli WP2 uvr A
Metabolic activation	with and without
Test system	all strains/cell types tested
Genotoxicity	negative
Cytotoxicity	no (Tested up to limit concentrations recommended by the test guideline)
Vehicle controls valid	yes
Positive controls valid	yes

Additional information on results

RANGE-FINDING STUDY: - Results on solubility: dimethyl disulphide formed a soluble and clear solution in dimethyl sulfoxide (DMSO) at approximately 500 mg/mL, the highest concentration tested.- Results on cytotoxicity: In the initial toxicity-mutation assay, the maximum dose tested was 5000 µg/per plate; this dose was achieved using a concentration of 100 mg/mL and a 50 µL plating aliquot. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate. Neither precipitate nor appreciable toxicity was observed.

Any other information on results incl. tables

Table 1: Number of revertants per plate (first experiment) (mean of 2 plates)

	TA 98		TA 100		TA 1535		TA 1537		WP2 uvrA	
Conc. [µg/plate]	- MA	+MA	- MA	+ MA	- MA	+ MA	- MA	+MA	-MA	+MA
DMSO	17	19	126	127	16	16	9	5	11	12
1.5	21	21	122	131	14	13	6	10	7	13
5.0	15	21	101	137	14	18	7	8	8	13
15	19	21	103	142	14	21	2	7	10	9
50	14	24	103	124	10	14	7	6	10	14
150	13	20	95	120	14	17	4	8	11	9
500	20	32	139	137	20	18	9	2	13	12
1500	17	19	136	131	17	7	4	7	10	10
5000	14	19	103	138	16	25	7	8	12	13

Positive control	170	553	426	604	424	70	389	68	52	87

Table 2: Number of revertants per plate (second experiment) (mean of 3 plates)

	TA 98		TA 100		TA 1535		TA 1537		WP2 uvrA	
Conc. [µg/plate]	- MA	+MA	- MA	+ MA	- MA	+ MA	- MA	+ MA	- MA	+ MA
DMSO	24	30	119	134	28	15	8	11	14	16
50	18	29	110	137	24	11	6	6	11	13
150	25	29	118	131	32	16	6	5	14	14
500	22	29	113	122	23	14	6	6	14	18
1500	19	34	107	126	26	15	7	6	15	20
5000	21	29	113	121	30	18	6	7	16	18
Positive control	147	458	628	572	478	78	497	53	96	181

Applicant's summary and conclusion

Interpretation of results : negative

Conclusions

AIl criteria for a valid study were met. The results of the Bacterial Reverse Mutation Assay indicate that, under the conditions of this study, Dimethyl disulphide did not cause a positive response in either the presence or absence of Aroclor-induced rat liver S9.

Executive summary

Dimethyl disulfide, was tested in the Bacterial Reverse Mutation Assay using*Salmonella typhimurium*tester strains TA98, TA100, TA1535 and TA1537 and*Escherichiacoli*tester strain WP2*uvrA*in the presence and absence of Aroclor-induced rat liver S9. The assay was performed using the plate incorporation method.The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate in the initial toxicity-mutation assay and50, 150, 500, 1500 and 5000 µg per plate in the confirmatory mutagenicity assay. No positive mutagenic response was observed. Neither precipitate nor appreciable toxicity was observed. Dimethyl disulfide was concluded to be negative in the Bacterial Reverse Mutation Assay

[Study 2] Jones (HRC) 1985/K1 SS/Ames test

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	Supporting Stduy	1 (reliable without restriction)	Jones E	1985	Ames metabolic activation test to assess thepotential mutagenic effct of dimethyl disulphide

Materials and methods

Type of genotoxicity : gene mutation

Type of study : bacterial reverse mutation assay (e.g. Ames test)

Test guideline

Qualifier	Guideline	Deviations
according to	EU Method B.13/14 (Mutagenicity - Reverse Mutation Test Using Bacteria) (1984)	

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 98.98%

Method

Target gene : Histidine

Species/strain

Species/strain	S. typhimurium, other: TA98, TA 100, TA1535, TA1537, TA 1538
Details on mammalian cell lines (if applicable)	Strains were properly maintained and were checked for appropriate genetic markers (rfa mutation, R factor)
Metabolic activation	with and without
Metabolic activation system	S9 derived from Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice.

Test concentrations

(a) Preliminary cytotoxicity assay: Plate incorporation assay: 0, 5, 50, 500 and 5000 µg per plate were evaluated with and without S9 activation in all strains. A single plate was used, per dose, per condition.
(b)Mutation assays: Plate incorporation assay: 50, 150, 500, 1500 and 5000 µg per plate were evaluated in triplicate in the presence and absence of S9 activation; all test strains were used.

Vehicle : DMSO

Controls

Negative controls	yes (Culture medium)
Solvent / vehicle controls	yes (DMSO)
True negative controls	no
Positive controls	yes
Positive control substance	other: With S9 mix: 2-Aminoanthracene (2 µg/plate) for TA 1535, TA 1537, TA 1538, TA 98 and TA 100. Without S9 mix: 2-nitrofluorene (10 µg/plate) for TA 1538 and TA98; 9-aminoacridine (20µg/plate) for TA 1537 and sodium azide (5µg/plate) for TA1535 and TA 100.

Details on test system and conditions

PRELIMINARY TOXICITY ASSAY
The preliminary toxicity assay was used to establish the dose range over which the test article would be assayed.
MUTAGENICITY ASSAY
Five dose levels of test article along with appropriate vehicle control and positive controls were plated with overnight cultures of TA98, TA100, TA1535, TA1537 and TA1538 on selective agar in the presence and absence of Aroclor induced rat liver S9. All dose levels of test article, vehicle control and positive controls were plated in triplicate. - Second mutation test: none
TEST PROCEDURE
- Without metabolic activation 0.1 ml aliquots of bacterial suspension and 0.5 ml of sterile 0.1 M sodium phosphate buffer (pH 7.4) are added to each of one set of sterile bijou bottles. 0.1 ml of the test compound is added to cultures at five concentrations separated by half-log 10 intervals. The negative control is the chosen solvent. The appropriate

positive control is also included. 3 bottles are used at each dose level. 2.0 ml of histidine deficient agar is added to each of the bottles, thoroughly mixed and then overlaid onto previously prepared plates containing 15 ml of minimal aaar. Plates are incubated for 72 hours at 37°C. Colonies are counted using a Biotran Automatic Colony Counter, and the mean number of revertant colonies per treatment group assessed. - With metabolic activation Methodology is as described above except that 0.5 ml of liver homogenate S-9 mix is added to bijou bottles in place of sterile buffer.

Evaluation criteria

The mean number of revertant colonies for all treatment groups is compared with those obtained for negative and positive control groups. The effect of metabolic activation is assessed by comparing the results obtained both in the presence and absence of the liver microsomal fraction for each treatment group. A compound is deemed to provide evidence of mutagenic potential if (1) a statistically significant dose-related increase in the number of revertant colonies is obtained in two separate experiments, and (2) the increase in the number of revertant colonies is at least twice the concurrent solvent control value.

Results and discussions

Test results

Species/strain	S. typhimurium, other: TA 1535, TA 1537, TA 1538, TA 98, TA 100
Metabolic activation	with and without
Test system	all strains/cell types tested
Genotoxicity	negative
Cytotoxicity	no (Tested up to limit concentrations recommended by the test guideline)
Vehicle controls valid	yes
Negative controls valid	not examined
Positive controls valid	yes

Any other information on results incl. tables

Table 1: Number of revertants per plate (first experiment) (mean of 3 plates)

	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
Conc. [µg/plate]	- MA	+MA	- MA	+ MA	- MA	+ MA	- MA	+MA	- MA	+MA
5000	7	8	6	9	4	9	13	13	62	67
1500	8	5	9	10	8	10	13	13	71	71
500	10	8	9	10	6	8	14	14	63	75
150	13	10	8	13	8	10	12	14	67	71
50	12	10	9	7	6	10	14	16	62	76
0	11	14	12	7	6	7	13	19	65	71
Solvent	9	7	7	12	6	8	16	16	63	75
Positive control	681	77	71	52	209	381	257	334	589	432

Table 2: Number of revertants per plate (second experiment) (mean of 3 plates)

	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
Conc. [µg/plate]	- MA	+MA	- MA	+ MA	- MA	+ MA	- MA	+MA	- MA	+MA
5000	10	9	8	12	7	13	19	18	57	59
1500	9	13	18	19	10	15	17	20	66	69
500	10	9	14	12	8	8	16	18	64	78
150	11	9	14	13	8	8	15	22	68	82
50	13	12	13	12	8	12	16	24	78	93
0	12	13	17	15	9	12	16	15	73	93
Solvent	11	8	15	12	6	8	17	16	91	92
Positive control	596	91	151	69	158	432	191	332	542	590

Applicant's summary and conclusion

Interpretation of results : negative

Conclusions

Dimethyl disulphide did not show any mutagenic activity in the bacterial reverse mutation test with Salmonella typhimurium.

Executive summary

The potential of dimethyl disulphide to induce reverse mutation in*Salmonella typhimurium* (strains: TA 1535, TA 1537, TA 1538, TA 98 and TA 100) was evaluated in accordance with the international guidelines (Commission Directive No. B13/14). Dimethyl disulphide was tested in two independent experiments, with and without a metabolic activation system, both performed by direct incorporation. Bacterias were exposed to dose-levels of 50, 150, 500, 1500 and 5000 µg per plate (three plates/dose-level) selected from a preliminary toxicity test. After 72 hours of incubation at 37°C, the revertant colonies were scored. Dimethyl disulphide did not induce any increase in the number of revertants, both with and without S9 mix, in any of the five strains.

[Study 3] Barfknecht (PRI) 1985/K2 SS/Ames test

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	Supporting Stduy	Barfknecht TR	1985	DIMETHYL DISULFIDE, Ames Salmonella/Microsome Plate Test (EPA/OECD)	

Materials and methods

Type of genotoxicity : gene mutation

Type of study : bacterial reverse mutation assay (e.g. Ames test)

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 471 (Bacterial Reverse Mutation Assay) (1983)	yes (The study was not repeated)

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: no data

Method

Target gene : Histidine

Species/strain

Species/strain	S. typhimurium, other: TA 1535, TA 1537, TA 1538, TA 98, TA 100
Details on mammalian cell lines (if applicable)	Test organisms were properly maintained and were checked for appropriate genetic markers (rfa mutation, R factor)
Metabolic activation	with and without
Metabolic activation system	S9 derived from Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice.

Test concentrations

(a) Preliminary cytotoxicity assay: Plate incorporation assay: 0, 50, 144, 500, 1444 and 5000 µg per plate were evaluated without S9 activation with strains TA100 and TA 1538. Two plate was used, per dose, per condition.
(b) Mutation assays: Plate incorporation assay: 0, 50, 166, 500, 1666 and 5000 µg per plate were evaluated in triplicate in the presence and absence of S9 activation; all test strains were used.

Vehicle : DMSO

Controls

Negative controls	yes (culture medium)
Solvent / vehicle controls	yes (Dimethylsulphoxide)
True negative controls	no
Positive controls	yes
Positive control substance	other: With S-9 mix: 2-Aminoanthracene (5 µg/plate), TA 1535, TA 1537, TA 1538, TA 98 and TA 100. Without S-9 mix: 2-Nitrofluorene (5 µg/plate) TA 1538 and TA98, 9-Aminoacridine (150 µg/plate) TA 1537, sodium azide (10 µg/plate) TA 1535 and TA 100.

Details on test system and conditions

PRELIMINARY TOXICITY ASSAY
The preliminary toxicity assay was used to establish the dose range over which the test article would be assayed.
MUTAGENICITY ASSAY
- Five dose levels of test article along with appropriate vehicle control and positive controls were plated with overnight cultures of TA98, TA100, TA1535, TA1537 and TA1538 on selective agar in the presence and absence of Aroclor induced rat liver S9. All dose levels of test article, vehicle control and positive controls were plated in triplicate.
- Second mutation test
The procedure was repeated at a later date.
TEST PROCEDURE
- Without metabolic activation 0.1 ml aliquots of bacterial suspension is added to each of one set of sterile tubes. 0.1 ml of the test compound is added to cultures at five concentrations. The negative control is the chosen solvent. The appropriate positive control is also included. 3 bottles are used at each dose level. 2.0 ml of histidine deficient agar is

added to each of the bottles, thoroughly mixed and then overlaid onto minimal glucose plates. Plates are incubated for 48-72 hours at 37°C. Colonies are counted using an Automatic Colony Counter, and the mean number of revertant colonies per treatment group assessed.
- With metabolic activation
Methodology is as described above except that 0.5 ml of liver homogenate S-9 mix is added to the tubes in place of sterile buffer.

Evaluation criteria

The mean number of revertant colonies for all treatment groups is compared with those obtained for negative and positive control groups. The effect of metabolic activation is assessed by comparing the results obtained both in the presence and absence of the liver microsomal fraction for each treatment group. A compound is deemed to provide evidence of mutagenic potential if (1) a statistically significant dose-related increase in the number of revertant colonies is obtained in two separate experiments, and (2) the increase in the number of revertant colonies is at least twice the concurrent solvent control value.

Results and discussions

Test results

Species/strain S. typhimurium, other:

Metabolic activation with and without

Test system other: TA 1535, TA 1537, TA 1538, TA 98, TA 100

Genotoxicity negative

Cytotoxicity no (Tested up to limit concentrations recommended by the test guideline)

Vehicle controls valid yes

Negative controls valid yes

Positive controls valid yes

Any other information on results incl. tables

Number of revertants per plate (first experiment) (mean of 3 plates)

	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
Conc. [µg/plate]	- MA	+MA	- MA	+ MA	- MA	+ MA	- MA	+ MA	-MA	+MA
DMSO	19	11	18	19	16	28	30	41	170	205
50	147	17	16	21	19	31	33	47	183	224
166	17	11	16	19	19	29	38	43	164	197
500	16	13	17	21	19	31	25	52	171	204
1666	13	13	13	25	16	30	38	39	169	197
5000	13	15	11	19	27	28	34	40	143	147
Positive control	689	245	1124	406	482	956	520	2168	753	928

Applicant's summary and conclusion

Interpretation of results : negative

Conclusions

Dimethyl disulphide was negative in the Ames/Salmonella tester strains TA1535, TA1537, TA1538, TA98 and TA100 with and without metabolic activation preparation over the dose range 50-5000 µg/plate.

Executive summary

The potential of dimethyl disulphide to induce reverse mutation in*Salmonella typhimurium* (strains: TA 1535, TA 1537, TA 1538, TA 98 and TA 100). Dimethyl disulphide was tested in a single experiment, with and without a metabolic

activation system, both performed by direct incorporation. Bacterias were exposed to dose-levels of 50, 166, 500, 1666 and 5000 µg per plate (three plates/dose-level) selected from a preliminary toxicity test. After 48 hours of incubation at 37°C, the revertant colonies were scored. Dimethyl disulphide did not induce any increase in the number of revertants, both with and without S9 mix, in any of the five strains.

[Study 4] De Vogel (CIVO-TNO) 1990/K1 KS/in vitro chromosomal aberrations, Human lymphocytes

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	Key Stduy	1 (reliable without restriction)	De Vogel N	1990	Chromosome analysis of cultured human|lymphocytes following in vitro treatment with DMDS

Materials and methods

Type of genotoxicity : chromosome aberration

Type of study : in vitro mammalian chromosome aberration test

Test guideline

Qualifier	**Guideline**	**Deviations**
according to	OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.88%

Method

Species/strain

Species/strain primary culture, other: Human Lymphocytes

Metabolic activation with and without

Metabolic activation system S9 derived from adult male Wistar rats (Aroclor 1254 induced rat liver).

Test concentrations :
0; 3.7; 11.1; 33.3; 100; 300 µg/ml

Vehicle : The test article (dissolved in Dimethyl sulfoxide (DMSO)) was soluble in culture medium at a maximum concentration of 1 mg/mL

Controls

Negative controls yes (culture medium)

Solvent / vehicle controls yes (DMSO)

True negative controls no

Positive controls yes

Positive control substance	other: Without S9: mitomycin C (MMC) 0.05 µg/mL. With S9: cyclophosphamide (CP) 25 µg/mL

Details on test system and conditions

- Preliminary Cytotoxicity Assay: The dose levels used in the chromosome aberration assay were established on the basis of the results of a preliminary toxicity test carried out with 6 concentrations of the test substance (ranging from 0.5 to 1000.0 µg/ml), both in the absence and in the presence of the metabolic activation system (S-9 mix). The highest concentration for the toxicity test was determined by the limit of the solubility of the test substance in the tissue culture medium (RPMI 1640 medium supplemented with heat-inactivated foetal calf serum, 100 units penicillin/mL, 100 µg streptomycin/mL, 2 mM L-glutamine and 25 µl phytohaemagglutinin/ml)
- Cytogenetic Assay:
* Cell Treatment After 48 h of incubation, the cultures were centrifuged at 800 rpm (100 g) and the supernatant removed. The cell pellets were resuspended in 4.5 ml tissue culture medium supplemented with 20 mM HEPES (and 10% S-9 mix, for the test with metabolic activation) and containing 50 µl of the appropriate test solutions. The final concentrations of the test substance in the culture medium were: 0.5, 1.4, 4.1, 12.3, 37.0, 111.1, 333.3 and 1000.0 µg/ml.An untreated culture and a culture receiving 50 µl of DMSO served as negative controls. For each concentration of the test substance and for the controls one culture was used. Without S9, the cultures were incubated in closed tubes for another 24 hours including a 2 hour colcemid treatment at 37°C in humidified air containing 5% CO2. With S-9 mix, the exposure of the cells to the test substance was reduced to only 2 hours, because of the toxicity of the S-9 mix for the cells. After the 2 hour incubation period, the cultures were centrifuged, the supernatant removed, the cells washed with phosphate- buffered saline (pH 7.4) and subsequently supplied with 4.5 ml freshly prepared culture medium. The cells were incubated for a further 22 hours (including a 2 hour colcemid treatment.
* Cell harvesting: Two hours before the end of the total incubation period the cells were arrested in the metaphase stage of the mitosis by the addition of colcemid (final concentration: 0.1 µg/ml medium). The cells were harvested by low speed centrifugation, treated for 15 minutes at 37°C with a hypotonic solution (0.075 M KCl), fixed three hours with a 3:1 mixture of methanol and glacial acetic acid, and transferred to clean microscope slides. Two slides were prepared from each culture. The slides were stained for 10 minutes in a 2% solution of Giemsa, rinsed in water, dried and mounted in DePeX. In each culture 1000 stimulated lymphocytes were examined (500 from each slide) to determine the mitotic index (percentage of cells in mitosis
* Metaphase analysis: From each culture, 100 well-spread metaphases (each containing 46 chromosomes) were analysed by microscopic examination for a wide range of structural chromosome aberrations (gaps, breaks, fragments, dicentrics, exchanges etc.) and other anomalies (endoreduplication, polyploidy), according to the criteria recommended by Savage (1975).

Evaluation criteria

The major criterion to designate the results of a chromosome aberration test as positive is a dose related, statistically significant increase in the number of cells with structural chromosome aberrations. However, a clear dose response relationship can be absent because the yield of chromosome aberrations can vary markedly with post treatment sampling time of an asynchronous population and because increasing doses of clastogens can induce increasing degrees of mitotic delay. A test substance producing neither a dose related, statistically significant increase in the number of cells with structural chromosome aberrations, nor a statistically significant and reproducible positive response at any of the doses is considered non-clastogenic in this system.

Statistics

Fischer's exact probability test.

<u>Results and discussions</u>

Test results

Species/strain	primary culture, other: Human Lymphocytes
Metabolic activation	with and without
Genotoxicity	ambiguous
Cytotoxicity	yes (clearly toxic at >= 300 µg/ml)
Vehicle controls valid	yes
Negative controls valid	yes

Positive controls valid yes

Additional information on results

Dimethyl disulphide did not induce a statistically significant increase in the number of cells with structural chromosome aberrations at non toxic concentrations, both in the absence and in the presence of the S-9 mix. At the very toxic concentration of 300.0 µg/ml, both in the absence and in the presence of the S-9 mix, the test substance induced a statistically significant increase in the number of cells with structural chromosome aberrations. The positive control substances, mitomycin C and cyclophosphamide, induced the expected increase in the incidence of structural chromosome aberrations.

Any other information on results incl. tables

Chromosomal aberrations in cultured human lymphocytes cells treated *in vitro* with DMDS in the absence of S-9 mix

Treatment	Dose (µg/ml)	N	Number of cells with aberrations				% cells with aberrations		Mitotic index (%)
			gaps	breaks	exchanges	multiple	Incl. gaps	Excl. gaps	
Control (medium)	-	200	0	2	0	0	1.0	1.0	4.5
Control (DMSO)	-	200	1	2	0	0	1.5	1.0	2.7
DMDS	3.7	200	4	1	0	0	2.5	0.5	3.7
	11.1	200	4	6	0	0	5.0	3.0	2.6
	33.3	200	7	3	0	0	5.0	1.5	3.4
	100.0	200	3	5	1	0	4.5	3.0	7.5
	300.0	200	15**	36***	1	1	23.5***	18.5***	0.7
Mitomycine C	0.05	200	6	25***	18***	0	21.5***	18.5***	5.2

Chromosomal aberrations in cultured human lymphocytes cells treated *in vitro* with DMDS in the presence of S-9 mix

Treatment	Dose (µg/ml)	N	Number of cells with aberrations				% cells with aberrations		Mitotic index (%)
			gaps	breaks	exchanges	multiple	Incl. gaps	Excl. gaps	
Control (medium)	-	200	0	1	0	0	0.5	0.5	4.3
Control (DMSO	-	200	1	2	1	0	2.0	1.5	5.4
DMDS	3.7	200	0	0	0	0	0.0	0.0	3.8
	11.1	200	3	5	0	0	3.0	2.5	4.8
	33.3	200	1	4	0	0	2.5	2.0	4.5
	100.0	200	9	6	3	0	8.5*	4.5	2.8
	300.0	200	13**	25***	10*	0	20.0***	16.0***	2.7
Cyclophosphamide	25	200	9	41***	38***	0	35.5***	33.3***	2.1

* p 0.05, ** p 0.01, *** p 0.001

Applicant's summary and conclusion

Interpretation of results : ambiguous (positive at cytotoxic concentrations, both with and without metabolic activation)

Conclusions

Dimethyl disulphide (DMDS) did not induce structural chromosome aberrations in cultured human lymphocytes at non toxic concentrations but it can mot be excluded that DMDS can react as a clastogen at very toxic concentrations, both in the absence and in the presence of the S-9 mix, under the conditions used in the present assay.

Executive summary

The potential of dimethyl disulphide (DMDS) to induce structural chromosome aberrations in human lymphocytes was evaluated according to OECD guideline in compliance with the Principles of Good Laboratory Practice. DMDS was

tested in one experiment, with and without a metabolic activation system. The lymphocytes cultures were exposed to positive and negative controls or DMDS at concentrations of 3.7, 11.1, 33.3, 100 and 300 µg/ml. The cultured cells were exposed for 2 hours with S9 mix and continuously until harvest without S9 mix. All cells were harvested 24H after initiation of the treatment. DMDS did not induce structural chromosome aberrations in cultured human lymphocytes at non toxic concentrations but it can not be excluded that DMDS can react as a clastogen at very toxic concentrations, both in the absence and in the presence of metabolic activation.

[Study 5] Rutten (CIVO-TNO) 1990/K1 KS/CHO HGPRT assay

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	Key Stduy	1 (reliable without restriction)	Rutten AA	1990	In Vitro assay for the induction of point mutations in the HGPRT-locus of Chinese hamster ovary cells by dimethyldisulfide (DMDS)

Materials and methods

Type of genotoxicity : gene mutation

Type of study : mammalian cell gene mutation assay

Test guideline

Qualifier	**Guideline**	**Deviations**
according to	OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.88%

Method

Target gene : HGPRT locus

Species/strain

Species/strain	Chinese hamster Ovary (CHO)
Metabolic activation	with and without
Metabolic activation system	S9 derived from adult male Wistar rats (Aroclor 1254 induced rat liver)

Test concentrations

First assay : 0.46, 1.37, 4.12, 12.3, 37.0, 111, 333 and 1,000 mg/lSecond assay: 4.12, 12.3, 37.0, 74.1, 111, 333, 667 and 1,000 mg/l

Vehicle : DMDS (dissolved in DMSO) was soluble in culture medium at a maximum concentration of 1 mg/mL

Controls

Negative controls	yes (culture medium)
Solvent / vehicle controls	yes (DMSO)
True negative controls	no
Positive controls	yes
Positive control substance	other: Without S9: Ethylmethanesulfonate 0.2 ml/L . With S9: Dimethylnitrosamine 2 or 4 ml/L.

Details on test system and conditions

The dose levels used in the HGPRT assay were established on the basis of the results of a preliminary solubility test. A final concentration of 1,000 µg/ml was chosen as highest concentration for the HGPRT assays. For the HGPRT-assay, aliquots of 1.8 x 10e6 CHO tells were added to a number of 75 cm2 tissue culture flasks containing 10 ml Ham's F-12 growth medium. The cells were incubated for approximately 20 hours in a humidified incubator at 37°C in air containing 5% CO2. On the day following seeding, the cells were exposed to the test substance, both in the absence and in the presence of a metabolic activation system (S-9 mix). In the absence of the S-9 mix, the cells were exposed to the test substance for 3 hours according to the following procedure. On the day of exposure, the tissue culture medium was removed and replaced by 9.9 ml Ham's F-12 containing gentamicin (50 mg/l) and L-glutamine (2 mM). One hundred µl of each of the test solutions were added to the culture medium. The final concentrations of the test substance in the culture medium selected for the exposure of the cells were:
a) first assay : 0.46, 1.37, 4.12, 12.3, 37.0, 111, 333 and 1,000 mg/l,
b) second assay: 4.12, 12.3, 37.0, 74.1, 111, 333, 667 and 1,000 mg/l.
In the presence of the S-9 mix, the cells were exposed to the test substance according to the following procedure. On the day of exposure, the tissue culture medium (Ham's F-12 medium supplemented with 10% heat-inactivated foetal calf serum, 50 µg gentamicin/mL and 2 mM L-glutamine) was removed and replaced by Ham's F-12 culture medium containing gentamicin (50 mg/1), L-glutamine (2 mM) and 10% (v/v) S-9 mix. The final concentrations of the test substance in the culture medium selected for the exposure of the cells were: a) first assay : 0.46, 1.37, 4.12, 12.3, 37.0, 111, 333 and 1,000 mg/l, b) second assay: 4.12, 12.3, 37.0, 74.1, 111, 333, 667 and 1,000 mg/l. For each concentration of the test substance and for the controls, one culture was used. After the 3-hour incubation period at 37°C the medium was removed, the cells were washed with phosphate-buffered saline (pH 7.4) and supplied with 10 ml growth medium. Subsequently, the cultures were incubated for an additional 18-21 hours in a humidified incubator at 37°C in air containing 5% C02. Cytotoxicity of the test substance was determined by measuring the colony-forming ability (cloning efficiency) of the CHO cells after the treatment period in the two independent HGPRT assays. The two independent HGPRT-assays were carried out with single cultures for each concentration of the test substance and for the negative and positive controls.

Evaluation criteria

The following criteria were used to evaluate the data obtained in the HGPRT assay (Li et al. 1987) a) the survival (absolute cloning efficiency) of the negative controls should not be less than 50%, b) the mean mutant frequency of the negative controls should fall within the range of 0-20 6-TG resistant mutants per 10e6 clonable cells, c) the positive controls must induce a response of a magnitude appropriate for the mutagen under the experimental conditions applied, d) the highest test substance concentration should, if possible, result in a clear cytotoxic response (e.g. 10-30% of the relative initial survival). Any apparent increase in mutant frequency at concentrations of the test substance causing more than 90% toxicity is considered to be an artifact and not indicative of genotoxicity. Genotoxicity of the test substance was evaluated using the following criteria (Li et al. 1987): a) a concentration-related increase in mutant frequency, b) a reproducible positive response for at least one of the test substance concentrations (e.g. the mean mutant frequency should be more than 20 mutants per 10e6 clonable cells).

Statistics

Exact statistical analysis is difficult because the distribution of the number of mutant colonies depends on assumptions of homogeneous variance, normal distribution, and the complex processes of cell growth and cell death after treatment with a test substance or a positive control (Li et al. 1987). Therefore, evaluation of the data obtained with the HGPRT assay was made on a case by case basis using the above described criteria, rather than by statistical analysis.

Results and discussions

Test results

Species/strain	Chinese hamster Ovary (CHO)
Metabolic activation	with and without
Test system	other: HGPRT assay on CHO cells
Genotoxicity	ambiguous
Cytotoxicity	yes (74.0-1000 µg/ml)
Vehicle controls valid	yes
Negative controls valid	yes
Positive controls valid	yes

Additional information on results

The actual concentrations of DMDS in culture medium were much lower than the target concentrations. Recovery experiments showed that about 50% of DMDS was lost directly on incubation (presumably by evaporation). Furthermore, during incubation an additional amount of 25% of DMDS is lost (presumably reactions with constituents of the incubation). In the absence of a metabolic activation system, the highest concentration (1,000 mg/l) showed an increased mutant frequency, only in the first HGPRT assay. At that concentration the test substance was highly toxic to the cells: the absolute initial cloning efficiency was reduced to about 22%. Furthermore, at this concentration droplets attached to the bottom of the tissue culture flask were observed. At lower concentrations (667, 333, 111 and 74.0 mg/l) which were still highly toxic, and at non-toxic concentrations (37.0 mg/l and lower), the mutant frequency did not differ clearly from that of the negative controls, in both independent assays. In view of these observations, the non-reproducible increase of the mutant frequency, at the highest concentration used, is not considered to be of biological significance. In the presence of a metabolic activation system, slight increases in mutant frequency were observed at several concentrations, both in the first (0.46, 12.3, 37.0, and 1,000 mg/l) and in the second assay (1,000 and 667 mg/l). Such increases occurred both at clearly toxic concentrations (absolute initial cloning efficiency of about 20%) and at non-toxic concentrations of the test substance. The increases in mutant frequency were not concentration-related.The positive control substances, EMS (in the absence of the S-9 mix) and DMN (in the presence of the S-9 mix), showed the expected increases in mutant frequency.

Any other information on results incl. tables

Results of the first assay:

Treatment	Dose (mg/l)	Mean relative initial cloning efficiency (%)		Mean absolute final cloning efficiency (%)		Mutant frequency per 10^6clonable cells	
		-S9	+S9	-S9	+S9	-S9	+S9
Control medium		100.0	100	82.0	75.8	4.9	12.5
Control DMSO		111.3	97.9	63.1	82.0	13.1	4.3
DMDS	0.46	108.5	94.1	80.4	87.8	2.5	22.8
	1.37	104.9	99.5	84.1	93.6	14.3	2.7
	4.12	111.9	101.6	75.1	77.8	8.7	7.1
	12.3	115.0	95.5	75.9	78.0	19.1	22.4
	37.0	102.2	100.5	75.9	81.2	15.2	30.2
	111	16.5	12.7	64.1	53.8	15.0	3.7
	333	33.3	29.0	54.3	81.8	0.0	3.7
	1000	28.1	17.7	73.9	81.9	82.6	25.6
EMS	0.2 ml/l	79.0	-	74.8	-	276.1	-
DMN	2.0 ml/l	-	51.5	-	75.1	-	45.9

Results of the second assay:

Treatment	Dose (mg/l)	Mean relative initial cloning efficiency (%)		Mean absolute final cloning efficiency (%)		Mutant frequency per 10^6clonable cells	
		-S9	+S9	-S9	+S9	-S9	+S9
Control medium		100	100	100.3	78.7	1.5	3.8
Control DMSO		115.2	83.3	106.5	77.9	4.7	7.7
DMDS	4.12	101.0	101.2	90.6	65.0	11.6	0.0
	12.3	111.0	102.5	85.5	77.8	9.9	3.2
	37.0	111.0	92.1	76.2	100.5	10.5	10.0

	74.1	35.3	19.2	85.0	89.5	2.9	2.8
	111	9.7	10.5	70.5	60.0	22.7	0.0
	333	17.1	23.4	63.4	76.7	0.0	17.6
	667	21.1	23.8	68.6	93.0	0.0	39.8
	1000	26.4	1.4	78.7	77.3	0.0	21.3
EMS	0.2 ml/l	93.9	-	73.4	-	180.5	-
DMN	2.0 ml/l	-	38.3	-	73.1	-	110.8
DMN	4 ml/l	-	9.5	-	65.8	-	171.7

Overall remarks, attachments

Historical negative control (vehicle) data from studies in 1989-1990.

Metabolic activation	Mutant frequency per 10^6 clonable cells mean ± standard deviation; range (number of assays)		
	medium	DMSO	tragacanth
without S9-mix	4.0 ± 2.9 0.0-8.3 (8)	8.9 ± 5.9 4.7-13.1 (2)	1.7 ± 0.8 1.1- 2.3 (2)
with S9-mix	3.7 ± 3.7 0.5-12.5 (8)	6.0 ± 2.4 4.3-7.7 (2)	1.3 ± 1.1 0.5- 2.0 (2)

Positive controls

Historical positive control data from studies in 1989-1990

Metabolic activation	Compound	Mutant frequency per 10^6 clonable cells mean ± standard deviation; range (number of assays)	
without S9-mix	EMS# 200 µg/l	147 ± 83	51-276 (6)
	EMS 200 µg/ml	63 ± 33	40-87 (2)
with S9-mix	DMN# 2 ml/l	80 ± 49	38-164 (7)
	DMN 4 ml/l	165 ± 111	54-348 (5)

#EMS = ethyl methanesulphonate
DMN = dimethylnitrosamine

Applicant's summary and conclusion

Interpretation of results

negative without metabolic activation

ambiguous with metabolic activation (positive at cytotoxic concentrations)

Conclusions

No conclusive evidence for a genotoxic effect of DMDS was found in cultured CHO cells, under the conditions used in the HGPRT assay.

Executive summary

Dimethyldisulfide (DMDS) was examined for its potential to induce point mutations in the HGPRT-locus of cultured

Chinese hamster ovary (CHO)cells, both in the absence and in the presence of a metabolic activationsystem (S-9). The test was conducted in compliance with OECD guideline 476. The dose levels used in the HGPRT assay were established on the basic of the results of a preliminary solubility test. Both in the absence and in the presence of a metabolic activation system (S-9 mix), the cells were exposed for 3 hours to 8concentrations of DMDS: 0.46, 1.37, 4.12, 12.3, 37.0, 111,333 and 1,000 mg/l in the 1stassay and 4.12, 12.3, 37.0, 74.0,111, 333, 667 and 1,000.0 mg/l in the 2ndassay. Ethylmethanesulfonate (in the absence of the S-9 mix) and dimethylnitrosamine (in the presence of the S-9 mix) were used as positive controls, while the vehicle (DMSO) and culture medium served as negative controls.
In the absence of the S-9 mix, DMDS induced neither a concentration-related increase in the mutant frequency nor areproducible positive response at one of the test concentrations. In thepresence of a metabolic activation system, DMDS induced a slight increase in mutant frequency at several concentrations, in both HGPRT assays. These increases were neither concentration-related nor clearly reproducible. In both HGPRT assays, the test substance appeared to behighly toxic to CHO cells at a concentration range from 74.0-1,000 mg/l. The actual concentrations ofDMDSin culture medium were much lower thanthe target concentrations. The positive control substances induced the expected increase in the mutant frequency.
No conclusive evidence for a genotoxic effect ofDMDSvas found in cultured CHO tells, under the conditions used in the HGPRT assay

[Study 6] Bichet (SANOFI) 1990/K1 KS/in vitro UDS assay

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	Key Stduy	1 (reliable without restriction)	Bichet N	1990	In Vitro DNA Repair Test on Rat Hepatocytes in Primary Culture

Materials and methods

Type of genotoxicity : DNA damage and/or repair

Type of study : DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells in vitro

Test guideline

Qualifier	**Guideline**	**Deviations**
according to	OECD Guideline 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro)	

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.88%

Method

Species/strain

Species/strain primary culture, other: Rat hepatocytes

Metabolic activation not applicable

Test concentrations

* Cytotoxicity studies:
 - 1st study : 1 - 5 - 10 - 100 and 200 µg/ml
- 2nd study : 5 - 10 - 50 - 100 - 150 - 200 - 250 and 300 µg/ml

* Genotoxicity studies:
- 1st study: 1- 5 - 10- 50 - 100 and 200 µg/ml
- 2nd study : 1 - 10 - 50 - 100 - 200 and 300 µg/ml

Vehicle : Dimethyl sulfoxide (DMSO). DMDS was soluble in culture medium at a maximum concentration of 100 µg/mL.

Controls	
Negative controls	yes (culture medium)
Solvent / vehicle controls	yes (DMSO)
True negative controls	yes (Pyrene 1 µM)
Positive controls	yes
Positive control substance	other: 7,12-DMBA (10 µM) and 2-aminofluorene (0.1 and 0.5 µM)

Details on test system and conditions

- Cytotoxicity evaluation: The test compound cytotoxicity was assessed for both DNA repair studies at the end of the treatment:
. by optical microscopic observation of the cell cultures,
. by measurement of the lactate dehydrogenase (LDH) activity.
- Incubation: Each concentration of Dimethyldisulfide was tested in triplicate. After 18 to 20 hours in a 95% air and 5% C02 humidified 37°C incubator, hepatocytes were washed with 2 ml of WME and observed under a microscope. Each coverslip was then washed with WME and immersed in 2 ml of 1% hypotonic sodium citrate, inducing a swelling of nuclei and a better quantification of nuclear grains. Finally, the cells were fixed in three 30-minute changes of ethanol and acetic acid (3:1), air-dried and mounted cell surface up on glass slides.
- Autoradiography: Autoradiographs were prepared by dipping slides in a photographic emulsion then developed. Slides were stained in hematoxylin-phloxin.
- Slide assessment: Grain courts were performed using an Artek electronic counter, connected to a microscope. For each cell, following nuclear grain court, cytoplasmic count was performed on 3 areas of the same size as the nucleus and adjacent to it.

Evaluation criteria

The test compound is considered positive when the mean nuclear grain court is statisticaly greater than that of the control, the mean net nuclear grain court is above 3 grains per nucleus, and the percentage of treated cells in repair is significantly different from that of the controls. In addition, the effect must be shown to be reproducible between experiments.

Statistics

Not appropriate

Results and discussions

Test results	
Species/strain	primary culture, other: Rat hepatocytes
Metabolic activation	not applicable
Genotoxicity	negative
Cytotoxicity	yes >= 100 µg/ml. IC50 evaluated by LDH release: 98 µg/ml (2nd study)
Vehicle controls valid	yes
Negative controls valid	yes
Positive controls valid	yes

Additional information on results

In both studies, the mean incorporation of tritiated thymidine in the nuclei of cells treated with DMDS at concentrations of 50, 100 and 200 µg/ml, was similar to that observed in control cells (solvent or untreated), and was lower than that observed in cytoplasms.The positive controls (DMBA and 2-AF), run in parallel induced incorporation of many nuclear grains at both concentrations, thus indicating an intense DNA repair synthesis due to a very active metabolism of hepatocytes, while pyrene control induced rates of incorporation of tritiated thymidine similar to chose obtained in control cells (solvent or untreated).

Any other information on results incl. tables

DNA Repair - First Study – Mean results

		Slide1	Slide2	Slide 3	Mean
Cellular control	NGC	12.7	12.3	14.3	13.1(±0.3)
	CGC	17.0	16.2	18.9	17.4(±0.5)
	NG	- 4.3	- 3.9	- 4.6	- 4.3(±0.5)
	% IR	0	0	0	0
DMSO	NGC	13.5	13.9	14.3	13.9 (±0.3)
	CGC	19.5	20.3	19.2	19.6(±t0.4)
	NG	- 6.0	- 6.4	- 4.9	- 5.7 (±0.4)
	% IR	0	0	0	0
Pyrene 1 µM	NGC	13.1	13.7	16.9	14.5 (±0.5)
	CGC	17.0	18.3	21.6	18.9 (±0-5)
	NG	- 3.9	- 4.6	- 4.7	- 4.4 (±0.5)
	% IR	0	0	0	0
DMBA 10 MM	NGC	34.6	35.7	36.6	35.6 (± 1.1)
	CGC	8.0	7.0	11.1	8.7 (± 0.4)
	NG	26.6	28.7	25.5	26.9 (±1.1)
	% IR	100	100	100	100
2-AF 0.5 uM	NGC	74.7	75.6	84.6	78.3 (± 1.7)
	CGC	15.4	18.6	18.6	17.5 (± 0.4)
	NG	59.3	57.0	66.0	60.8 (± 0.9)
	% IR	100	100	100	100
DMDS 200 µg/ml	NGC	11.6	21.3	17.1	16.6 (± 0.7)
	CGC	11.8	29.0	22.7	21.1 (± 1.0)
	NG	- 0.2	- 7.7	- 5.6	- 4.5 (±0.7)
	% IR	10	0	0	3
DMDS 100 µg/ml	NGC	20.7	16.7	14.7	17.4 (±0.6)
	CGC	26.7	25.2	25.2	25.7 (±0.7)
	NG	- 6.0	- 8.5	-10.5	- 8.3 (±0.6)
	% IR	0	0	0	0
DMDS 50 µg/ml	NGC	15.9	15.3	14.9	15.4 (±0.4)
	CGC	20.9	19.5	21.1	20.5(±0.4)
	NG	- 5.0	- 4.2	- 6.2	-5.1(±0.5)
	% IR	0	10	0	3

DNARepair - Second Study - Mean results

		Slide 1	Slide 2	Slide 3	Mean
Cellular control	NGC	20.0	21.3	28.0	23.1 (±0.7)
	CGC	24,1	29.1	34.6	29.3 (±0.7)
	NG	-4.1	- 7.8	- 6.6	- 6.2 (±0.5)
	% IR	3	0	0	0
DMSO	NGC	21.1	22.3	21.8	21.7 (±0.5)
	CGC	28.9	29.7	29.9	29.5 (±0.5)
	NG	-7.8	- 7.4	- 8.1	- 7.8 (±0.6)
	% IR	0	0	0	0
Pyrene 1 µM	NGC	16.4	18.0	17.8	17.4 (±0.5)
	CGC	22.6	23.1	22.9	22.9 (± 0.4)
	NG	-6.2	- 5.1	- 5.1	- 5.5 (± 0.5)
	% IR	0	0	0	0
2 AF	NGC	133.4	121.0	108.4	120.9 (±3.0)

0.5 µM	CGC	26.7	22.4	18.3	22.4 (±0.9)
	NG	106.7	98.6	90.1	98.5(± 2.8)
	% IR	100	95	100	98.3
2-AF 0.1µM	NGC	84.9	86.1	66.4	79.1 (±2.2)
	CGC	18.8	22.8	15.8	19.1 (±0,6)
	NG	66.1	63.3	50.6	60.0 (±2.0)
	% IR	100	100	100	100
DMDS 100 µg/ml	NGC	15.4	23.2	26.6	21.7 (±0.8)
	CGC	21.3	30.2	35.3	28.9(±0.9)
	NG	- 5.9	- 7.0	- 8.7	- 7.2 (±0.6)
	% IR	0	0	0	0
DMDS 50 µg/ml	NGC	19.0	20.9	16.5	18.8 (±0.6)
	CGC	24.9	23.3	23.4	23.9 (±0.5)
	NG	- 5.9	- 2.4	- 6.9	-5.1(±0.6)
	% IR	0	0	0	0
DMDS 10 µg/ml	NGC	19.3	16.4	17.1	17.6 (±0.5)
	CGC	28.1	28.8	24.5	27.1 (±0.6)
	NG	- 8.8	-12.4	- 7.4	- 9.5 (±0.7)
	% IR	0	0	0	0

NGC : Nuclear Grain Count
CGC: Cytoplasmic Grain Count
NG : Net Grain
%IR : Percentage of cells in repair (NG > 3)

Applicant's summary and conclusion

Interpretation of results : negative

Conclusions

DMDS did not induce DNA repair synthesis in the in vitro DNA repair assay at concentrations of 10, 50, 100 and 200 µg/ml throughout 2 independent studies

Executive summary

In a study performed according to the OECD Guideline #482, the potential genotoxicity of dimethyl disulphide (DMDS) was assessed*in vitro*on freshly isolated rat hepatocytes by comparing the incorporation oftritiated thymidineintreated cells with that induced in cells exposed to the solvent (dimethylsulfoxide or DMSO) or to genotoxic agents 2-aminofluorene and 7,12-dimethylbenz(a)anthracene).
DMDS was dissolved in DMSO and diluted at concentrations ranging front 1 to 30mg/ml. A precipitationof the compound in the culture medium at concentrations from 200 µg/ml upwards was observed.
DMDS was found to be cytotoxic at concentrations of 200, 250 and 300 µg/ml. Cytotoxicity was evaluated by measuring the release of lactate deshydrogenase, which gave an IC50 value of 98 µg/ml. BothDNArepair studies performed at concentrations of 10, 50, 100 and 200 µg/ml did not reveal any induction of unscheduledDNAsythesis in rat hepatocyte primary cultures exposed DMDS. In conclusion, DMDS was not genotoxic to rat hepatocytes in primary culture.

3.10.2 In vivo

[Study 1] Weinberg (WIL) 2007/K1 KS/Micronucleus assay in rats

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**

study report	Key Stduy	1 (reliable without restriction)	Weinberg JT	2007	Mammalian erythrocyte micronucleus assay of DMDS in rats

Materials and methods

Type of genotoxicity : chromosome aberration

Type of study : micronucleus assay

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	no
according to	EPA OPPTS 870.5395 (In Vivo Mammalian Cytogenics Tests: Erythrocyte Micronucleus Assay)	no

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.2%

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Laboratories, Raleigh, North Carolina, USA
- Age at study initiation: 9-week old
- Weight at study initiation: 280 g to 354 g for males and from 200 g to 238 g for females
- Assigned to test groups randomly: yes, using a computerized randomization procedure
- Fasting period before study: no
- Housing: individually in suspended wire-mesh cages
- Diet (ad libitum): PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002
- Water (ad libitum): municipal water
- Acclimation period: 7 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 21.3 to 21.7
- Humidity (%): 33.3 to 43.7
- Air changes (per hr): no data
- Photoperiod (hrs dark / hrs light): 12/12

IN-LIFE DATES: From 14 November 2006 to 13 December 2006

Administration / exposure

Route of administration : inhalation: vapour

Details on exposure

TYPE OF INHALATION EXPOSURE: whole body

GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION
- Exposure apparatus: Exposures were conducted in two 500-L whole-body chambers for the 0 and 825 ppm groups and two 130-L whole-body chambers for the 217 and 421 ppm groups.- Method of holding animals in test chamber: in wire mesh batteries containing separate cages,
- System of generating vapours: Vapors of the test article were generated using a 500-mL bubbler-type vaporization system located within a containment cart that was exhausted through the facility exhaust system. Individual gas washing bottles were wrapped in aluminum foil to protect the test article from light exposure. Nitrogen was metered into the inlet stem of the gas washing bottle containing an appropriate quantity of test article and bubbled through a fritted disc and the liquid test article to produce vapors of the test article An Advanced Specialty Gas Equipment rotameter-type flowmeter (Middlesex, NJ) for the 0 ppm group, Gilmont rotameter-type flowmeters (Barrington, IL) for the 217 and 421 ppm groups and Omega rotameter-type flowmeter (Stamford, CT) for the 825 ppm group were used to meter nitrogen to the gas washing bottles. The test article vapors were piped to the chamber inlet where the concentration was reduced to the desired concentration by mixing with the chamber supply air.
- Temperature, humidity, pressure in air chamber: Chamber temperature(24°C), relative humidity (35-56%) , ventilation rate and negative pressure within the chamber were recorded at approximately 30-minute intervals during the exposure.
- Air change rate: at least 10 air changes per hour- Oxygen content: 20.6% for the 0 ppm group and 20.9% for the 217, 421 and 825 ppm groups.
- Treatment of exhaust air: The exhaust atmosphere passed through the in-house exhaust system before being released from the facility.
TEST ATMOSPHERE
- Brief description of analytical method used: Analyzed exposure concentrations were determined at approximately 30-minutes intervals using a gas chromatograph (GC). Samples of the exposure atmosphere were collected from the chamber using an internal gas-sampling valve and sample loop. The chromatograph was displayed and the area under the sample peak was calculated and stored. The concentration in parts per million (ppm) was calculated using a ln-quadratic formula based on the GC calibration curve.
- Samples taken from breathing zone: yes

Duration of treatment / exposure : 4-hour exposure

Frequency of treatment : Single exposure

Post exposure period : up to 48 hours after inhalation exposure

Doses / concentrations : 217, 421, and 825 ppm

Basis analytical conc.

No. of animals per sex per dose : 10, control and high dose. 5, low and mid doses and positive control

Control animals : yes, sham-exposed

Positive control(s)

- Cyclophosphamide
- Justification for choice of positive control(s):
- Route of administration: intraperitoneal injection 24 hours prior to bone marrow collection
- Doses : 40 mg/kg

<u>Examinations</u>

Tissues and cell types examined : Bone marrow

Details of tissue and slide preparation

CRITERIA FOR DOSE SELECTION: The high test article exposure level was the maximum tolerated dose that did not induce mortality. This level was selected based on the results ofprevious acute toxicity studies with Dimethyl Disulfide.
SAMPLING TIMES:24 and 48 hours after the exposureDETAILS OF SLIDE PREPARATION:Bone marrow was collected from the femurs of each animal and transferred to a labeled centrifuge tube containing approximately 3 mL of fetal bovine serum. The bone marrow cells were centrifuged and the supernatant fraction was drawn off, leaving fetal bovine serum with the remaining cell pellet. The cells were resuspended by aspiration with a capillary pipette and a small drop of the cell suspension was spread onto a clean glass slide. Each slide was identified by the experiment and animal number. At least 2 slides were prepared from each animal. Bone marrow smears were fixed in methanol, air

dried and packed for transfer to BioReliance (Rockville, MD).
METHOD OF ANALYSIS: At BioReliance, slides were coded by using a random number table and stained with acridine-orange. Using a fluorescent microscope and medium magnification, an area of acceptable quality was selected such that the cells were well spread and stained. Using oil immersion, 2000 polychromatic erythrocytes (PCEs) were scored per animal for the presence of micronuclei (micronucleated PCEs, MPCEs). The number of micronucleated normochromatic erythrocytes in the field of 2000 polychromatic erythrocytes were enumerated, but not used to evaluate the response of the test article. The proportion of polychromatic erythrocytes to total erythrocytes was recorded per 1000 erythrocytes in test article-treated animals, which should not be less than 20% of the control value. The mean incidence of micronucleated polychromatic erythrocytes could not exceed 5/1000 polychromatic erythrocytes (0.5%) in the negative control. The incidence of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes for each animal and per 10,000 PCEs per each treatment group were determined and presented.

Evaluation criteria

The test article was considered to induce positive response if a dose-responsive increase in the incidence of micronucleated polychromatic erythrocytes was observed and one or more exposure levels were statistically elevated relative to the negative control ($p < 0.05$, Kastenbaum-Bowman Tables) at any sampling time.Values that were statistically significant but did not exceed the range of historical negative controls were judged as not biologically relevant.The test article was judged negative if no statistically significant increase in the incidence of micronucleated polychromatic erythrocytes above the concurrent negative control values and no evidence of dose response were observed at any sampling time.

Statistics

Kastenbaum-Bowman Tables

<u>Results and discussions</u>

Test results

Sex	male/female
Genotoxicity	negative
Toxicity	yes (Clinical signs and reductions in the ratio of PCEs to total erythrocytes)
Vehicle controls valid	yes
Negative controls valid	not examined
Positive controls valid	yes

Additional information on results

MORTALITY
None of the animals died during exposure or the post-exposure observation period.
CLINICAL OBSERVATIONS
During the animal exposure, clinical observations consisted of hypoactivity for all animals in the 217, 421 and 825 ppm groups; lacrimation for 1 male and 1 female in the 217 ppm group, 3 males in the 421 ppm group and 4 males and 3 females in the 825 ppm group; clear material around the mouth for 2 females in the 217 ppm group, 2 males and 3 females in the 421 ppm group and 3 males and 6 females in the 825 ppm group and red material around the nose for 1 male in the 217 ppm group. Clinical observations immediately following the exposure consisted of yellow material urogenital area for 1 female in the 217 ppm group, 2 females in the 421 ppm group and 3 females in the 825 ppm group; yellow material ventral neck for 3 females in the 825 ppm group; yellow material ventral trunk for 2 females in the 825 ppm group; red material around the nose for 1 male in the 421 ppm group and red material facia1 area and yellow material left forelimb for 1 male in the 217 ppm group. The only clinical observation at the post-exposure observation period was red material around the nose for 1 male in the 0 ppm group and 1 female in the positive control group.
BODY WEIGHTS
There were no effects on mean body weight (relative to the control group) for any group at 24 or 48 hours following exposure. For males and females euthanized 24 hours after exposure, the mean body weights for the 217, 421 and 825 ppm groups and the positive control group were 1 to 14 grams less than the control group. For animals euthanized 48 hours after exposure, the mean body weight for the 825 ppm group was 23 and 7 grams less than the control group values for males and females, respectively. This suggests a slight body weight loss for animals in the test article-exposed groups and the positive control group.
BONE MARROW ANALYSIS

A statistically significant increase in the number of micronucleated polychromatic erythrocytes was not observed in the test article-exposed groups relative to the negative control group. In addition, reductions in the ratio of PCEs to total erythrocytes, up to 5%, were observed in some test article-exposed groups relative to the negative control group, suggesting that the test article did not inhibit erythropoiesis. CP induced a significant increase in the incidence of micronucleated polychromatic erythrocytes in both male and female rats, indicating that the bone marrow erythropoietic cells were exposed to CP and that CP, a known clastogen, induced formation of micronuclei. In addition, the number of micronucleated PCEs did not exceed 5/1000 PCEs scored in the negative control group.

Any other information on results incl. tables

Summary of bone marrow micronucleus assay after a single 4-hour inhalation exposure of Sprague-Dawley rats to dimethyl disulphide

Treatment	Sex	Time (hr)	# of animals	PCE/total erythrocytes (mean±SD)	MPCE/1000PCE (mean±SD)
Negative control, 0 ppm (air-only)	M F	24 24	5 5	0.626±0.06 0.649±0.03	0.1±0.22 0.2±0.27
Dimethyl disulphide212 ppm	M F	24 24	5 5	0.637±0.04 0.583±0.03	0.2±0.27 0.1±0.22
Dimethyl disulphide425 ppm	M F	24 24	5 5	0.600±0.03 0.626±0.03	0.1±0.22 0.0±0.00
Dimethyl disulphide850 ppm	M F	24 24	5 5	0.629±0.03 0.611±0.04	0.5±0.50 0.7±0.57
Positive control Cyclophophamide, 40 mg/kg	M F	24 24	5 5	0.459±0.07 0.421±0.03	25.1±2.16 11.2±1.82
Negative control, 0 ppm (air-only)	M F	48 48	5 5	0.616±0.02 0.617±0.05	0.5±0.50 0.0±0.00
Dimethyldisulphide850 ppm	M F	48 48	5 5	0.634±0.02 0.580±0.04	0.1±0.22 0.0±0.00

Applicant's summary and conclusion

Interpretation of results : negative

Conclusions

Dimethyl Disulfide at concentrations of 217, 421 and 825 ppm did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythocytes in the bone marrow when male and female albino rats were exposed to test article as a single, 4-hour, whole-body inhalation exposure.

Executive summary

In a micronucleus assay performed following the OECD guideline # 474 and the OPPTS Guideline # 870.5395 dimethyl disulphide at concentrations of 217, 421 and 825 ppm did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythocytes in the bone marrow when male and female Sprague-Dawley rats were exposed to test article as a single, 4-hour, whole-body inhalation exposure.

[Study 2] Willems (CIVO-TNO) 1989/K1 SS/micronucleus assay in mice

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Supporting Stduy	1 (reliable without restriction)	Willems MI	1989	Examination of dimethyl disulfide in the micronucleus test,

Materials and methods

Type of genotoxicity : chromosome aberration

Type of study : micronucleus assay

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.88%

Test animals

Species : mouse

Strain : Swiss

Sex :
male/female

Details on test animals and environmental conditions
TEST ANIMALS:
- Source: Charles River France SA, Saint Aubin les Elboeuf, France
- Age at study initiation : no data
- Body weight at study initiation: no data
- Acclimation period: 6 days
ENVIRONMENTAL CONDITIONS:
- Housing: individually in Makrolon cages
- Diet: Institute's cereal baszed, open formula diet
- water: tap water ad libidum
- Temperature: 22 ± 2°C,
- Humidity: 40 to 60%,
- Air changes: about 10 cycles/hour
- Photoperiod: 12 h/12 h

Administration / exposure

Route of administration : inhalation

Vehicle(s) none

Details on exposure
TYPE OF INHALATION EXPOSURE: whole body
GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION
- Exposure chambers Animals were exposed in horizontally placed glass tube inhalation chambers with a capacity of 15 l. The all glass construction of the chamber enabled observation of the animals during exposure. The total air flow through the chambers varied approximately between 13 and 15.5 l/min.
- Generation of the test atmosphere The test atmospheres were generated by passing adjustable flows of filtered air from the compressed air system through evaporation vessels filled with DMDS. The vessels with DMDS were kept at a temperature of approximately 25°C. The DMDS laden airflow was passed to the mixing device of the inhalation chambers where it was diluted with make-up air. The make up air was obtained from the compressed air system.

Before mixing with the test material the air was filtered and moisturized to enhance the relative humidity. Next the test atmospheres were led to the inlet of the inhalation chambers.
TEST ATMOSPHERE
- Test atmosphere control During the exposures the concentration of the test material was determined approximately once or twice each hour by means of a gas chromatograph. The gas chromatograph was fitted with a flame ionization detector (FID). The nominal concentrations were calculated for each concentration level each day.

Duration of treatment / exposure : 4 days

Frequency of treatment : 6 h/day

Post exposure period : None

Doses / concentrations : 0 , 250 and 500 ppm

Basis analytical conc.

No. of animals per sex per dose : Three groups of mice were exposed during 6 hours a day for 4 consecutive days (days 0 through 3) to atmospheres containing 0 ppn (5/sex), 250 ppm (5/sex) and 500 ppm DMDS (10/sex).

Control animals : yes, sham-exposed

Positive control(s)

The positive control group (5/sex) was treated once intraperitoneally, 24 hours before sacrifice, with 1.5 mg Mitomycin C per kg body weight.

Examinations

Tissues and cell types examined : Bone marrow

Details of tissue and slide preparation

Animals were killed in the morning of day 4, except for the surviving animals of the 500 ppm group which were killed on day 3 in view of their bad condition. Bone marrow cells were collected from the femur and processed into smears for microscopic examination. One smear from each animal was examined for the presence of micronucleated poly- and normochromatic erythrocytes, (abbreviated MPE and MNE, respectively), and the total numbers of poly- and normochromatic erythrocytes (PE and NE) in a total of at least 2000 erythrocytes (E) in such a way that a minimum of 1000 PE was observed.

Evaluation criteria

Not specified

Statistics

Statistical analysis was carried out in two stages. In the first step of the procedure overall significance tests were carried out as follows: the fraction of MEP, MNE and ME per counted number of PE, NE, and E, respectively, were analysed with a generalized linear model using a binomial error-distribution (McCullagh and Nelder, 1983), and the numbers of PE per 1000 erythrocytes were analyzed with linear regression techniques (e.g. Draper and Smith, 1981). Both methods assess the influence of sex (male, female), treatment (vehicle control, test groups) and the various interactions on the total variation in the data. As a second stage, a posteriori comparisons of treatment groups with negative control were carried out with asymptotic t-tests if either the main effect of treatment or the treatment by sex interaction was significant. For all calculations involved, the Genstat 5 statistical package was used (Genstat 5 committee, 1987).

Results and discussions

Test results

Sex	male/female
Genotoxicity	negative
Toxicity	yes (Mean numbers of PE/1000 E were slightly lower in mice exposed to 500 ppm)
Vehicle controls valid	yes

Negative controls valid not applicable

Positive controls valid yes

Additional information on results

Exposure to DMDS resulted in clear signs of intoxication both at the 250 ppm and the 500 ppm level, and 12 (7 males, 5 females) out of the 20 mice of the 500 ppm group died. Exposure to 250 ppm and 500 ppm DMDS resulted in body weight loss both in males and females. There were no indications for increases in the incidences of MPE, MNE or ME attributable to treatment with the test material. Mean numbers of PE per 1000 E were slightly lower in mice exposed to 500 ppm DMDS, both in males and females (0.001<P<0.01) pointing to slight cytotoxic effects on bone marrow cells. Animals treated with the mutagen Mitomycin C showed an increased incidence of MPE.

Any other information on results incl. tables

Group mean numbers of micronucleated erythrocytes per 1000PE, 1000 NE and 1000 E, and the group mean numbers of PE per 1000 E

Treatment	Dose	PE per 1000 E	MPE per 1000 PE	MNE per 1000 NE	ME per 1000 E
MALES					
Control (n = 5)	0 ppm	500	0.6	1.2	0.8
DMDS (n = 5)	250 ppm	532	2.6*	0.8	1.7
DMDS (n = 3)	500 ppm	452**	1.7	1.3	1.4
Mitomycine (n = 5)	1.5 mg/kg	496	48.7***	2.0	25.4***
FEMALES					
Control (n = 5)	0 ppm	520	2.5	1.2	1.8
DMDS (n = 5)	250 ppm	540	1.0	0.9	1.0
DMDS (n = 5)	500 ppm	358**	1.0	0.7	0.8*
Mitomycine (n = 5)	1.5 mg/kg	487	41.0***	2.0	21.1***

Abbreviations used,
PE: polychromatic erythrocytes
NE: normochromatic erythrocytes
E: erythrocytes
MPE, MME, ME : micronucleated PE, ME, E, respectively
* 0.01 < P < 0.05
** 0.001 < P < 0.01
***P < 0.001

Applicant's summary and conclusion

Interpretation of results : negative

Conclusions

The results of the micronucleus test did not provide any indication of chromosomal damage and/or damage to the

mitotic apparatus in bone marrow cells of mice exposed to DMDS.

Executive summary

Dimethyl disulfide (DMDS) vas examined in the micronucleus assay in mice in compliance with the OECD guideline # 474. Three groups of mice were exposed during 6 hours a day for 4 consecutive daysto atmospheres containing 0 ppm (5/sex), 250 ppm (5/sex) and 500 ppm DMDS (10/sex).The positive control group (5/sex) was treated once intraperitoneally,24 hours before sacrifice, with 1.5 mg Mitomycin C per kg body weight.Animals were killed in the morning of day 4, except for the survivinganimals of the 500 ppm group which were killed on day 3 in view of their bad condition. Bone marrow cells were collected from the femurand processed into smears for microscopic examination. One smear fromeach animal vas examined for the presence of micronucleated poly- and normochromatic erythrocytes, (abbreviated MPE and MME, respectively),and the total numbers of poly- and normochromatic erythrocytes (PE andNE) in a total of at least 2000 erythrocytes (E) in such a way that aminimum of 1000 PE vas observed.
Exposure to DMDS resulted in clear signs of intoxication both at the250 ppm and the 500 ppm level, and 12 (7 males, 5 females) out of the20 mice of the 500ppmgroup died.Exposure to 250 ppm and 500 ppm DMDS resulted in body weight loss bothin males and females.There were no indications for increases in the incidences of MPE, MNE or ME attributable to treatment with the test material.
Mean numbers of PE per 1000 E were slightly lover in mice exposed to 500 ppm DMDS, both in males and females (0.00150.01) pointing to slight cytotoxic effects on

[Study 3] Rutten (CIVO-TNO) 1990/K1 KS/in vivo-in vitro UDS assay

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key Stduy	1 (reliable without restriction)	Rutten AA	1990	An in vivo/in vitro rat hepatocyte DNA-repair assay with dimethyldisulfide (DMDS)

Materials and methods

Type of genotoxicity : DNA damage and/or repair

Type of study : unscheduled DNA synthesis

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD guideline 482 (DNA Damage and Repair/Unscheduled DNA Synthesis in Mamnalian Cells in vitro)	
equivalent or similar to	OECD Guideline 486 (Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo) (The study was performed before the implementation of the guideline)	yes (No positive control was used for the early sampling times (2-4 hours). A single concentration was tested, but this concentration was the MTD)

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.88%

Test animals

Species : rat

Strain **:**
Wistar

Sex : male

Details on test animals and environmental conditions

TEST ANIMALS:
- Source: Charles River Wiga GmbH, Sulzfelfg, FGR
- Age at study initiation : no data
- Body weight at study initiation: 180-245 g
- Acclimation period: at least 6 days

ENVIRONMENTAL CONDITIONS:
- Housing: 5 per suspended stainless steel cages
- Diet: Institute's cereal baszed, open formula diet
- water: tap water ad libidum
- Temperature: 22 ± 2°C,
- Humidity: 30 to 70%,
- Air changes: about 10 cycles/hour
- Photoperiod: 12 h/12 h

<u>**Administration / exposure**</u>

Route of administration : inhalation

Vehicle(s) : None

Details on exposure

- Exposure chambers Animals were exposed in horizontally placed glass tube inhalation chambers with a capacity of 15 l. The all glass construction of the chamber enabled observation of the animals during exposure. The total air flow through the chambers varied approximately between 13 and 15.5 l/min.
- Generation of the test atmosphere

The test atmospheres were generated by passing adjustable flows of filtered air from the compressed air system through evaporation vessels filled with DMDS. The vessels with DMDS were kept at a temperature of approximately 25°C. The DMDS laden airflow was passed to the mixing device of the inhalation chambers where it was diluted with make-up air. The make up air was obtained from the compressed air system. Before mixing with the test material the air was filtered and moisturized to enhance the relative humidity. Next the test atmospheres were led to the inlet of the inhalation chambers.
- Test atmosphere control During the exposures the concentration of the test material was determined approximately once or twice each hour by means of a gas chromatograph. The gas chromatograph was fitted with a flame ionization detector (FID). The nominal concentrations were calculated for each concentration level each day.

Duration of treatment / exposure : 4 hours

Frequency of treatment : Single exposure

Post exposure period : 0, 16 or 24 hours

Doses / concentrations : 0 and 500 ppm

No. of animals per sex per dose

Negative control groups: n=5
DMDS-treated groups: n = 8
Positive control group: n = 4

Control animals : other: Hepatocytes isolated from animals exposed to air only served as negative controls.

Positive control(s)

The hepatocarcinogen 2-acetylaminofluorene (2 AAF: 50 mg/kg by gavage), was used as a positive control in the in vivo/in vitro DNA-repair assay and in the in vitro DNA-repair assay (2 AAF: final concentration of 10e-4 M in tissue culture medium).

Examinations

Tissues and cell types examined : Hepatocytes

Details of tissue and slide preparation

After in vivo exposure, hepatocytes were isolated from the liver of the rats using the perfusion technique described by Williams et al. (1977), Mitchell and Mirsalis (1984), and Butterworth et al. (1987), with minor modifications. Briefly, the liver of each rat was perfused in situ with a Ca- and Mg-free HEPES buffer (0.01 M), whilst under Nembutal anaesthesia. The in situ perfusion was followed by an in vitro perfusion with a HEPES-buffered (0.1 M) collagenase (500 mg/l) solution. After isolation, the dissociated cells were incubated in WEH medium [= Williams medium E supplemented with 20 mM HEPES buffer and bovine serum albumin (10 mg/ml)] and incubated for 15 minutes in a shaking waterbath at 37°C. Thereafter, the cells were filtered over a 100 mesh nylon filter, centrifuged again (2.5 min, 50 x g), resuspended in WEH medium, centrifuged and finally resuspended in WEC medium (= Williams medium E complete, which consists of Williams medium E supplemented with 10% fetal calf serum, B-glutamine (2 mM) and gentamicin (50 pg/ml)]. Cell counts were made with a haemocytometer. The viability of the hepatocytes was determined by trypan blue exclusion. Only hepatocyte suspensions with viability greater than 60% were used in the repair assay, except for the in vivo positive control group (2-AAF; 50 mg/kg).

* Butterworth, B.E., J. Ashby, E. Bermudez, D. Casciano, J. Mirsalis, G. Probst and G. Williams (1987) A protocol and guide for the in vivo rat hepatocyte DNA-repair assay. Mutation Research 189, 123-133.
* Mitchell, A.D. and J.C. Mirsalis (1984) Unscheduled DNA synthesis as an indicator of genotoxic exposure. In: Single cell mutation monitoring systems, (eds) A.A. Ansari and F.J. De Serres, Plenum Publishing Corporation, pp. 165-216.
* Williams, G.M. (1977) Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. Cancer Research 37, 1845-1851.

Evaluation criteria

The following criteria were used to evaluate the DNA-repair :
a) if the test substance yields greater than- or equal to 5 NG (population average) and,
b) greater than- or equal to 20% of the cells responding, the response is considered positive,
c) population average between 0 and 5 NG would be considered to be a marginal response.

Statistics

Body weights and plasma enzyme activities were evaluated by one-way analysis of variance (ANOVA) followed by Student's t-test (two-sided). Statistical analysis of the mean number of grains per nucleus was not done, because the data are not appropriate for analysis.

Any other information on materials and methods incl. tables

Dimethyldisulfide (DMDS) was examined for its potential to induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes after short-term exposure of male wistar rats to the test substance by inhalation.
In the preliminary toxicity tests, 20 maleWistar rats were allocated atrandomto four groups of 3 ratsand exposed by inhalationfor4 h,to thefollowingtarget concentrations of0 ppm, 100 ppm, 200 ppm, and 400 ppm DMDS. Clinicalsymptoms wereobservedatregular (1 h) intervals during the 4 h exposure period, directly after the 4 hexposure period, andat24 and 48 hafterterminationoftheexposure to DMDS. Bodyweights wereobservedjust before the start of the exposure, and at 20.5 and 40.5 h after terminationoftheexposure to DMDS. In view ofthe absenceof clear intoxication symptoms, a second toxicity test was performed at target concentrations of 0and 600 ppm DMDS. In this test, 20 maleWistar wereallocatedat random to two groups of10rats andexposed by inhalationfor4h to the target concentrations of0 ppm and600ppm DMDS. Clinical symptoms and bodyweights were observed asdescribedfor the first toxicity test, up to 22 h.In additionobservations weremadeofplasma glutamic-oxalacetic-transaminase (GOT) and glutamic-pyruvic-transaminase (GPT) activities todisclose possiblesigns of toxicity. Bloodwascollectedfrom 5 animals per groupdirectlyafter exposureandfrom the remaining 5 animals per group at24 hafterexposure, byaortapuncture whilstthe rats were under anesthesia.
In the genotoxiicty assay, groups of animals were exposed by inhalation for a single period of 4 h to0 ppm or 500 ppm DMDS. Immediately after exposure andafter non-exposure periods of 16 and 24 h respectively, animals were sacrificed for isolation of hepatocytes to be used in the in vitro DNA-repair assay.Clinical symptoms were examined at regular intervals (1 h) during the 4 hexposure period and directly after this period. Body weights were observedat 24 h before the exposure and just before the start of the exposure.Theenzyme activities of glutamic pyruvic transaminase (GPT) and gamma-glutamyl-transpeptidase (GGT) in plasma (blood collected from the tip ofthe tail) of 5 control rats and of 5 rats

exposed to 500 ppm DMDS was determined directly after the 4-h exposure period.

<u>Results and discussions</u>

Test results

Sex	male
Genotoxicity	negative
Toxicity	yes (Increased GGT enzyme activities in plasma of rats exposed to 500 ppm)
Vehicle controls valid	yes
Negative controls valid	not examined
Positive controls valid	yes

Additional information on results

1. Exposure concentrations
The actual concentrations were very close to the target concentrations (Table 1).
2. Toxicity testing
In the 1st toxicity test, clinical signs, observed at 1 h intervals during the 4-h exposure period to 100, 200 and 400 ppm DMDS, were mainly characterized by restless behavior, salivation, half closed and closed eyes, lachrymation, mouth breathing, labored and increased frequency of breathing, piloerection, coats soiled with urine, and serous nasal discharge. Furthermore, a slight decrease in body weights of the animals exposed to DMDS was observed 24 h after exposure. However, 48 h after exposure to DMDS (recovery period) mean body weights of all groups were increased.In the 2nd toxicity test, body weights and clinical symptoms were affected in a way similar to the findings observed with 400 ppm in the first toxicity test. Plasma enzyme activities were slightly increased directly after the 4 h exposure period, whereas 24 h after exposure, the GOT and GPT activities were similar to those of the unexposed controls. Slightly elevated levels of GOT and GPT activities were also observed after treatment of CD-1 mouse with dimethylnitrosamine, indicating slight hepatic toxicity.
3. DNA-repair assay
Gamma-glutamyl-transpeptidase (GGT) enzyme activities in plasma of rats exposed to 500 ppm DMDS were clearly increased directly after the 4-h exposure (as compared to the unexposed controls), indicating a slight hepatotoxic effect of DMDS. Clinical signs, during the 4-h exposure period to 500 ppm DMDS, were similar to observed in both toxicity tests.From the results obtained it appeared that inhalation exposure to the maximally tolerated concentration of 500 ppm DMDS for 4 h, did not induce DNA-repair in hepatocytes, either during the 4 h exposure period or during the subsequent 16 h or 24 h after the exposure period (Table 2). All test groups showed a DNA-repair activity within the variation of unexposed controls.Both in the in vivo/in vitro DNA-repair assay and in the in vitro DNA-repair assay the hepatocarcinogen, 2-acetylaminofluorene (2-AAF) showed the expected increase in DNA-repair activities. The induction of DNA-repair in the in vitro assay was more pronounced than in the in vivo assay.

Any other information on results incl. tables

Table 1. Target, nominal and actual concentrations of DMDS in the testatmospheres

DMDS (ppm)	Nominal concentration	Actual concentration[a)]
First toxicity test		
100	N.D[b)]	106.6 ± 5.2
200	N.D.	202.8 ± 7.8
400	N.D.	423.4 ±31.8
Second toxicity test		

600	644	583.6 ±26.2
DNA-repair assay		
500[c)]	477	508.8 ±17.3
500[d)]	515	495.7 ± 41.5
500[e)]	559	480.3 ±90.2

[a)]Mean concentrations of three samples at 1 h intervals.
[b)]Not determined
[c)]isolation hepatocytes directly after exposure
[d)]isolation hepatocytes 16 h after exposure
[e)]isolation hepatocytes 24hafter exposure

table 2: Results of the in vivo/in vitro DNA-repair assay with DMDS (summarized results for each dose level)

Treatment	Dose	Mean nuclear grain counts	Mean cytoplasm grain counts[a]	Mean net grains per nucleus	Cells in repair[b)](%)	Mean nuclear grains cells in repair	cells in S-phase(%)
Isolation of rat hepatocytes directly after exposure							
DMDS	0 ppm	13.52 ± 2.64[c)]	17.41 ± 4.84	-3.89 ± 2.21	6.00	7.77 ± 2.10	0.20
DMDS	500 ppm	15.14 ± 1.42	21.06 ± 1.35	-5.92 ± 0.72	5.00	8.51 ± 0.82	0.54
2-AAF (in vitro)	10^{-4}M	35.68 ± 9.93	9.15 ± 1.56	26.53 ± 8.37	93.33	28.06 ± 7.48	1.03
Isolation of rat hepatocytes 16 hours after exposure							
DMDS	0 ppm	14.53 ± 2.09	18.51 ± 3.11	-3.98 ± 1.00	6.00	8.43± 2.84	0.57
DMDS	500 ppm	14.37 ± 1.66	18.76 ± 2.35	-4.14 ± 1.12	5.50	7.62± 0.75	0.13
2-AAF (in vitro)	10^{-4}M	65.51 ±10.02	20.63 ± 1.08	44.88 ± 9.01	94.67	47.48 ± 9.98	1.70
Isolation of rat hepatocytes 24 hours after exposure							
DMDS	0 ppm	13.46 ± 4.18	17.17 ± 3.82	-3.72 ± 0.35	6.00	6.47 ± 0.55	0.70
DMDS	500 ppm	16.58 ± 1.90	22.66 ± 2.85	-6.08 ± 1.13	4.17	7.26 ± 1.64	0.07
2-AAF (in vitro)	10^{-4}M	53.21 ± 6.59	16.49 ± 3.91	36.71 ± 3.41	98.00	37.44 ± 2.82	0.83
Isolation of rat hepatocytes 18 hours after exposure							
2-AAF (in vivo)	50 mg/kg	37.34 ± 0.67	29.99 ± 4.01	7.40 ± 3.40	53.00	15.22 ± 2.85	1.23

a) Mean cytoplasm grain count of nuclear sized area
b) Percent of cells in repair (more or equal than 5 mean net grains per nucleus)
c) C) Standard deviation (animal to animal)

Applicant's summary and conclusion

Interpretation of results : negative

Conclusions

DMDS did not induce DNA-repair in hepatocytes of rats exposed to the test substance by inhalation, at the maximally tolerated concentration of 500 ppm.

Executive summary

Dimethyldisulphide (DMDS) was examined for its potential to induceunscheduled DNA synthesis (UDS) in primary rat hepatocytes after short-term exposure of male Wistar rats to the test substance by inhalation.The study wasconductedin compliance with OECD guideline # 482, and the recommendations byButterworth et al. (1987) for an*in vivo/in vitro*rat hepatocyte DNA-repair assay, which are comparable to the OECD guideline # 486. The toxicity of DMDS in rats was investigated by inhalation, for oneperiod of 4 h, at concentrations of 0, 100, 200 and 400 ppm (firsttoxicity test) and 0 and 600 ppm (second toxicity test). Exposureto high DMDS dose levels (400 or 600 ppm) resulted in body weightloss, increased plasma enzyme activities and increased incidence of clinical signs both with respect to behavior and physical condition.
For the genotoxicity assay male rats were exposed by inhalation for a period of 4 h to one high concentration of 500 ppm DMDS (maximally tolerated concentration). Immediately after exposure and aftersubsequent non-exposure periods of 16 and 24 h, animals were sacrificedfor isolation of hepatocytes. The DNA-repair activities were examined byautoradiography in monolayer cultures of hepatocytes, incubated in thepresence of [methyl-^{3}H]thymidine. The hepatocarcinogen 2-acetylaminofluorene (2-AAF: 50 mg/kg by gavage),was used as a positive control in the*in vivo/in vitro*DNA-repair assayand in the*in vitro*DNA-repair assay (2-AAF: final concentration of 10^{-4}M in tissue culture medium). Hepatocytes isolated from animals exposedto air only served as negative controls.
DMDS did not induce DNA-repair activities in hepatocytes, either duringthe 4 h exposure period or during the subsequent 16 h or 24 h after theexposure period.The positive control substance, 2-AAF, induced the expected increase inDNA-repair activities.It was concluded that DMDS did not induce DNA-repair in rat hepatocytes.

3.11 Reproductive toxicity

3.11.1 Toxicity to reproduction

[Study 1] Nemec (WIL) 2006/K1 KS/2-generation study

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key Stduy	1 (reliable without restriction)	Nemec MD	2006	A two-generation reproductive toxicity inhalation study of dimethyl disulfide in rats

Materials and methods

Test type : two-generation study

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)	no

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.8% (certificate of analysis at study initiation),
99.18% (certificate of analysis at study conclusion)

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Laboratories, Inc., Raleigh, North Carolina,
- Age at reception: 31 days old (F0)
- Age at the initiation of test article exposure: 6.6 weeks
- Body weight at exposure initiation: (F0) Males: 211-263 g; Females: 137-178 g
- Housing (non exposure periods): individually in wire-mesh cages suspended above cage-board. After mating and until weaning, the females were transferred to plastic maternity cages with nesting material.
- Diet : PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002, ad libitum. Food was withheld during the exposure periods.
- Water: municipal water, ad libitum. Water was withheld during the exposure periods.
- Acclimation period: 14 days

ENVIRONMENTAL CONDITIONS (NON-EXPOSURE PERIODS)
- Temperature (°C): 22 ± 3 (actual : 21.3°C to 21.5°C)
- Humidity (%): 50 ± 20 (actual: 35.7% to 62.1%)
- Air changes (per hr): 12
- Photoperiod (hrs dark / hrs light): 12/12

IN-LIFE DATES: From 2005-06-28 to 2006-06-09

Administration / exposure

Route of administration : inhalation: vapour

Type of inhalation exposure (if applicable) : whole body

Vehicle : unchanged (no vehicle)

Details on exposure

Exposures were conducted in 2.0-m3 stainless steel and glass whole-body exposure chambers. One chamber was dedicated to each group for the duration of the study. The exposure period was 6 hours per day, 7 days per week and was defined as the time between turning the generation system on and off, including the start equilibration time. The chambers were operated under dynamic conditions, at a slight negative pressure (ca 0.5 in of water) with at least 12 to 15 air changes per hour.Vapors of DMDS were generated using a bubbler-type (gas washing bottle) vaporization system. DMDS vapors were then directed to the exposure chamber inlet where vapor concentration was reduced to the desired level by mixing with the chamber ventilation air.

Details on mating procedure

The animals were paired on a 1:1 basis within each treatment group after a minimum of 70 days of exposure. All animals were randomly selected for pairing, avoiding sibling matings. Each female was housed in the home cage of the male. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm in a vaginal lavage. Each mating pair was examined daily. The day when evidence of mating was identified was termed gestation day 0. The animals were separated, and the female was housed in an individual plastic cage with nesting material. When evidence of mating was not apparent after 14 days, the female was placed in a plastic maternity cage with nesting material, with no further opportunity for mating.

Analytical verification of doses or concentrations : yes

Details on analytical verification of doses or concentrations

Actual exposure concentrations within each chamber were measured at least 10 times (approximately every 35 minutes) during each daily exposure period by a gas chromatographic method. At least 1 standard was analyzed each day prior to

exposure to confirm gas chromatographic calibration. Overall mean measured test article exposure concentrations for the F0 generation were 0, 5.0, 20.4 and 79.9 ppm and for the F1 generation were 0, 5.0, 20.6 and 80.0 ppm for the filtered air, 5, 20 and 80 ppm groups, respectively.

Duration of treatment / exposure

The F0 and F1 males and females were exposed to the test atmosphere for a minimum of 70 consecutive days prior to mating. Exposure of the F0 and F1 males continued throughout mating and through the day prior to euthanasia. The F0 and F1 females continued to be exposed throughout mating and gestation through gestation day 20. To prevent confounding effects on nursing, exposure for F0 and F1 females was suspended from gestation day 21 through lactation day 4, inclusively, and was re-initiated on lactation day 5 and continued through the day prior to euthanasia. During lactation (except when indicated above), the dams were removed from their litters during each daily 6-hour exposure period.

Frequency of treatment : 6 hours per day, 7 days per week

Details on study schedule

Each generation was mated once to produce 1 litter per generation (the F1 and F2 litters). Prior to the F0 pairing (week 10), male body weights ranged from 355 g to 623 g and female body weights ranged from 230 g to 363 g. The animals were approximately 17 weeks old. All animals were randomly selected for pairing. Each F0 dam and litter remained housed together until weaning on lactation day 28. Thirty male and 30 female F1 pups from each group (control, 5, 20 and 80 ppm) were randomly selected prior to weaning (PND 28) to comprise the F1 generation. These pups (a minimum of 1 male and 1 female per litter, when available) were exposed to the test article for 6 hours per day, 7 days per week beginning on PND 28.Prior to the F1 pairing (week 29), male body weights ranged from 300 g to 631 g and female body weights ranged from 203 g to 364 g. The animals were approximately 15 to 17 weeks old.

Doses / concentrations : 5, 20 and 80 ppm

Basis analytical conc .

No. of animals per sex per dose 30

Control animals : yes, sham-exposed

Positive control : Not appropriate

Examinations

Parental animals: Observations and examinations

CAGE SIDE OBSERVATIONS: Yes

All animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity. In addition, the animals were observed for appearance, behavior and pharmacotoxic signs at the midpoint of exposure for animals visible through the chamber windows and 1 hour after completion of exposure for all animals. Females expected to deliver were also observed twice daily during the period of expected parturition and at parturition for dystocia (prolonged labor, delayed labor) or other difficulties.

DETAILED CLINICAL OBSERVATIONS: Yes

Detailed physical examinations were recorded weekly for all parental animals throughout the study period.

BODY WEIGHT: Yes

Individual F0 and F1 male body weights were recorded weekly throughout the study and prior to the scheduled necropsy. Individual F0 and F1 female body weights were recorded weekly until evidence of copulation was observed. Once evidence of mating was observed, female body weights were recorded on gestation days 0, 4, 7, 11, 14, 17 and 20 and on lactation days 1, 4, 7, 14, 21 and 28. After weaning (lactation day 28), weekly body weights were recorded for these females until the scheduled necropsy.

FOOD CONSUMPTION: yes

Individual F0 and F1 male and female food consumption was measured weekly until pairing. Food intake was not recorded during the mating period. Following mating, male food consumption was measured on a weekly basis until the scheduled necropsy. Female food consumption was recorded on gestation days 0, 4, 7, 11, 14, 17 and 20 and lactation days 1, 4, 7, 14, 21 and 28.

WATER CONSUMPTION: No

Estrous cyclicity (Parental animals)

Vaginal lavages were performed daily and the slides were evaluated to assess the regularity and duration of the estrous cycles of each F0 and F1 female for 21 days prior to pairing and continuing until evidence of mating was observed or until the end of the mating period.

Sperm parameters (Parental animals)

Spermatogenic endpoints (sperm motility including progressive motility, morphology and numbers) were recorded for all surviving F0 and F1 males.

Litter observations

F0 AND F1 PARTURITION
All females were allowed to deliver naturally and rear their young to weaning (PND 28). During the period of expected parturition, the females were observed twice daily for initiation and completion of parturition and for signs of dystocia. Beginning on the day parturition was initiated (PND 0), pups were sexed and examined for gross malformations, and the numbers of stillborn and live pups were recorded. Individual gestation length was calculated using the date delivery started.
STANDARDISATION OF LITTERS
8 pups per litter, 4 per sex when possible, were randomly selected on PND 4. Standardization of litter size was not performed on litterswith fewer than 8 pups. All selections were performed by computerized randomization. The remaining offspring were weighed, euthanized by intraperitoneal injection of sodium pentobarbital and discarded on PND 4.
PARAMETERS EXAMINED IN F1 AND F2 LITTERS
- LITTER VIABILITY AND DEATHS
Each litter was examined twice daily for survival, and all deaths were recorded.
- CLINICAL OBSERVATIONS
Litters were examined daily for survival and any adverse changes in appearance or behavior. Each pup received a detailed physical examination on PND 1, 4, 7, 14, 21 and 28. Any abnormalities in nursing behavior were recorded.
- BODY WEIGHTS
Pups were individually weighed on PND 1, 4, 7, 14, 21 and 28.
- SEX DETERMINATION
Pups were individually sexed on PND 0, 4, 21 and 28.
- BALANOPREPUTIAL SEPARATION
Each male pup was observed for balanopreputial separation beginning on PND 35. Examination of the pups continued daily until balanopreputial separation was present. Body weights were recorded at the age of attainment of this landmark.
- VAGINAL PATENCY
Each female pup was observed for vaginal perforation beginning on PND 25. Examination of the females was continued daily until vaginal patency was present. Body weights were recorded at the age of attainment of this landmark.
GROSS EXAMINATION OF DEAD PUPS:yes,
for external and internal abnormalities; possible cause of death was not determined for pups born or found dead.

Postmortem examinations (Parental animals)

SACRIFICE
All F0 adults were euthanized following the selection of the F1 generation and completion of a detailed clinical observation. All surviving F1 adults were euthanized following weaning of the F2 pups.
GROSS NECROPSY
A complete necropsy was conducted on all parental animals (F0 and F1) found dead, euthanized in extremis or at termination. The necropsy included examination of the external surface, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, and the thoracic, abdominal and pelvic cavities, including viscera.For females that delivered or had macroscopic evidence of implantation, the numbers of former implantation sites (the attachment site of the placenta to the uterus) were recorded.
ORGAN WEIGHTS
The following organs were weighed from all F0 and F1 parental animals at the schedulednecropsies: Adrenal glands, Brain, Epididymidesa (total and cauda), Kidneys, Liver, Ovaries, Pituitary gland, Prostate gland, Seminal vesicles with coagulating glands (with accessory fluids), Spleen, Testes(a, Thymus gland, Uterus with oviducts and cervix.(a = These paired organs were weighed separately.

HISTOPATHOLOGY
Microscopic evaluations were performed on the following tissues for F0 and F1 parental animals from the control and high exposure groups and for all adult animals found dead or euthanized in extremis: Adrenal glands, Brain, Cervix, Coagulating gland, Epididymisa (right): caput, corpus and cauda, Kidneys, Liver, ovaries, Oviducts, Pituitary gland, Prostate gland, Seminal vesicles, Spleen, Testis (right), Thymus gland, Uterus, Vagina, Vas deferens, All gross (internal) lesions (all groups).Because the adrenal cortex was identified as a potential target tissue, all of the F0 male rats in the 5 and 20 ppm groups were examined in addition to the initial examination of the control and 80 ppm groups.

Postmortem examinations (Offspring)
SACRIFICE AND GROSS NECROPSY
All remaining nonselected F1 and F2 weanlings were euthanized and necropsied on PND 28, with emphasis on developmental and reproductive system morphology. All gross lesions from F1 and F2 weanlings and the brain, spleen and thymus from 1 randomly selected weanling per sex per litter were preserved in 10% neutral-buffered formalin for possible future histopathologic examination; all other tissues were discarded. The carcasses were then discarded.
ORGAN WEIGHTS
The following organs were weighed from 1 randomly selected pup per sex per litter for the nonselected F1 and F2 pups: Brain, Thymus, Spleen.

Statistics

Where applicable, the litter was used as the Parental mating and fertility indices were analyzed using the Chi-square test with Yates' correction factor. Mean parental (weekly, gestation and lactation) body weights and body weight changes, parental food consumption and food efficiency data, estrous cycle lengths, pre-coital intervals, gestation lengths, implantation sites, live litter sizes, unaccounted sites, numbers of pups born, balanopreputial separation data (day of attainment and body weight), vaginal patency data (day of attainment and body weight), absolute and relative organ weights, sperm production rates, epididymal and testicular sperm numbers, and ovarian primordial follicle counts, were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test article-exposed groups to the control group. Mean offspring body weights and body weight gains during PND 1-4 and 4-28 were analyzed separately by sex using an analysis of covariance (ANCOVA), with litter size as the covariate. Mean litter proportions (percent per litter) of postnatal pup survival and pup sexes at birth (percentage of males per litter), percentages of motile sperm, and percentages of sperm with normal morphology were subjected to the Kruskal-Wallis nonparametric ANOVA test to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunn's test was used to compare the test article-exposed groups to the control group. Histopathological findings in the test article-exposed groups were compared to the control group using a two-tailed Fisher's Exact test.

Reproductive indices

Mating, fertility, copulation and conception indices were calculated.

Offspring viability indices

Mean Live Litter Size, postnatal Survival Between Birth and PND 0 or PND 4 (Pre-Selection), postnatal Survival for All Other Intervals indices were calculated.

<u>Results and discussions</u>

Effect levels

Endpoint	**Generation**	**Sex**	**Effect level**	**Based on**	**Basis for effect level / Remarks**
NOAEC (reproductive and developmental toxicity)	other: F0 and F1	male/female	80 ppm (analytical)		No functional effects on reproduction (estrous cycles, mating and fertility indices, number of days between pairing and coitus, and gestation length) in any DMDS-exposed group. There were no adverse effects on pups born to dams exposed to DMDS and results from several studies confirm a lack of effect on postnatalgrowth prior to weaning with exposure of the lactating dams.
NOAEC (parental	other: F0	male/female	5 ppm		persistent decrements in mean body weights, body

systemic toxicity)	and F1		(analytical)		weight gains and/or food consumption, increase in the incidence of vacuolization of the adrenal cortex or increased adrenal gland weights

Results of examinations: parental animals

Clinical signs (parental animals) : no effects

Body weight and food consumption (parental animals) : yes

Reproductive function: estrous cycle (parental animals) : no effects

Reproductive function: sperm measures (parental animals) : no effects

Reproductive performance (parental animals) : no effects

Organ weights (parental animals) : yes

Gross pathology (parental animals) : no effects

Histopathology (parental animals) : yes

Details on results (parental animals)

F0 GENERATION
- CLINICAL SIGNS AND MORTALITY

All F0 parental animals in the control, 5, 20 and 80 ppm groups survived to the scheduled necropsy. No exposure-related clinical findings were noted during the F0 generation at the weekly examinations or at the midpoint exposure and 1 hour following exposure observations.
- BODY WEIGHT

* WEEKLY

Reduced (p<0.01) mean body weight gains were noted in the 20 and 80 ppm F0 male groups during study weeks 0-1 (31 g and 14 g, respectively) and 1-2 (40 g and 34 g, respectively) of test article exposure when compared to the control group values of 40 g and 47 g, respectively. Lower mean body weight gain in the 80 ppm F0 male group continued throughout the remaining pre- and post-mating exposure periods when compared to the control group; the differences from the control group values were statistically significant (p<0.05 or p<0.01) during study weeks 2-3, 7-8, 8-9, 9-10, 15-16 and 16-17. Mean body weight gain in the 20 ppm F0 male group was similar to the control group during the remaining pre- and post-mating exposure periods. As a result, cumulative mean body weight gains in the 80 ppm F0 male group were statistically significantly (p<0.01) reduced at 200 g and 258 g during the entire pre-mating (study weeks 0-10) exposure period and during the entire generation (study weeks 0-19), respectively, when compared to a mean body weight gains of 266 g and of 339 g, respectively, in the control group. The cumulative mean body weight changes in the 20 ppm F0 male group during these same exposure intervals were only slightly lower (not statistically significant) when compared to the control group and indicated partial recovery of body weight gain effects noted during the first 2 weeks of exposure.During the first week of exposure (study week 0-1), mean body weight gains in the 20 and 80 ppm group F0 females (17 g and 12 g, respectively; statistically significant, p<0.05 or p<0.01) were lower when compared to a mean body weight gain of 21 g in the control group. Statistically significant (p<0.05 or p<0.01) reduced mean body weight gains in the 80 ppm F0 female group continued during study weeks 1-2 and 2-3 when compared to the control group (18 g compared to 25 g and 24 g compared to 29 g, respectively). Mean body weight gains in the 20 ppm group were similar to the controlgroup from study weeks 1-2 and statistically significantly lower (p<0.05) during study weeks 2-3 (24 g compared to 29 g). These findings were attributed to the onset of test article exposure. Following the initial 3 weeks of exposure, mean body weight changes in the 20 and 80 ppm group females were similar to the control group during study weeks 3-10. The cumulative mean body weight gains in these respective groups were 144 g and 133 g, respectively, during the pre-mating period (study weeks 0-10) compared to 154 g in the control group. The differences from the control group were statistically significant (p<0.01) in the 80 ppm group and not statistically significant in the 20 ppm group.Accordingly, reductions in mean body weights (3.6% to 5.3% and 9.9% to 12.8%, respectively; p<0.05 or p<0.01) were observed in the 20 and 80 ppm F0 male groups during the first 3 weeks of exposure when compared to the control group. Statistically significantly lower (11.7% to 15.2%; p<0.01) mean body weights in the 80 ppm F0 male group continued from study week 4 throughout the remaining pre- and post-mating periods when compared to the control group. Mean body weights were only slightly reduced (3.2% to 4.4%; not statistically significant) in the 20 ppm F0 male group during this same interim compared to the control group. When the F0 females were evaluated at 80 ppm, reductions in mean body weights (6.1% to 9.5%; p<0.01) were observed during the entire treatment period when compared to the control group. In addition, mean body weights in the 20 ppm F0 female group were only slightly reduced (3.0% to 5.2%) during the exposure period compared to the control group; differences were statistically significant during study weeks 1 and 3 only. The reductions in mean body weights in the

80 ppm group, and to a lesser extent in the 20 ppm group, were due to exposure-related decrements in the rates of weight gains noted in the first 1 to 3 weeks of the study.No exposure-related effects on mean body weights or mean body weight gains were observed in the 5 ppm group males and females. Slightly lower mean body weight gains (p<0.05) were noted in these males during study weeks 1-2 and 16-17 when compared to the control group. These differences were not of sufficient magnitude to cause reductions in cumulative mean body weight gains from study weeks 0-10 and 0-19, and therefore, indicated no adverse effect at the lowest exposure concentration.
* GESTATION
No exposure-related effects in mean body weight change were noted in any exposure concentration during the F0 gestation period. Lower mean body weights (6.3% to 7.4%) in the 80 ppm group were observed during the entire gestation period when compared to the control group. The differences from the control group values were statistically significant (p<0.01) and were due to the lower mean body weight gain observed during the pre-mating period. Mean body weights in the 5 and 20 ppm groups were similar to the control values during this exposure period.
* LACTATION
Slightly lower mean body weights (4.8% to 7.4%) in the 80 ppm F0 female group were noted during lactation days 1-28 compared to the mean body weights in the control group; the differences from control values were statistically significant (p<0.05 or p<0.01). These decreased mean body weights were attributed to decreased mean body weight gain during the pre-mating period. No exposure-related effects were noted in mean body weights in the 5 and 20 ppm groups or in mean body weight changes in 5, 20 and 80 ppm during the lactation period when compared to the control group values.
- FOOD CONSUMPTION
* WEEKLY
Mean food consumption, evaluated as g/animal/day, was statistically significantly lower (p<0.05 or p<0.01) in the 20 and 80 ppm F0 male groups during study weeks 0-2 when compared to the control group. Mean food consumption, evaluated as g/kg/day, was also statistically significantly lower (p<0.01) in the 80 ppm male group during study weeks 0-1 and in the 20 and 80 ppm male groups (p<0.05 or p<0.01) during study weeks 1-2. These differences were related to the onset of exposure to the test article. Following the first 2 weeks of exposure, mean food consumption in the 20 ppm group males was unaffected by test article exposure, while statistically significantly (p<0.05 or p<0.01) lower mean food consumption, evaluated as g/animal/day, in the 80 ppm F0 males continued throughout the remaining exposure period. However, mean food consumption, evaluated as g/kg/day, in this same group were statistically significantly higher (p<0.01 or p<0.05) during study weeks 3-4, 4-5, 5-6, 6-7, 7-8, 12-13, 13-14, 14-15, 17-18 and 18-19 when compared to the control group. These differences correlated with the lower mean body weights observed in this same group during thisexposure interim and were attributed to test article exposure.Exposure-related effects on mean food consumption were noted to a lesser extent in the 20 and 80 ppm F0 females than the corresponding F0 male groups. Mean food consumption in the 20 ppm (g/animal/day) and 80 ppm (g/animal/day and g/kg/day), group F0 females were statistically significantly lower (p<0.01) during study week 0-1 when compared to the control group. Mean food consumption (g/animal/day) in the 80 ppm group continued to be lower (p<0.01) during study weeks 1-2 and 2-3. These differences were also related to the onset of exposure to the test article and correlatedwith the reduced mean body weight gain observed in these females during the first 2 weeks of exposure. Mean food consumption (g/animal/day) in the 20 and 80 ppm female groups was similar to the control group values during the remainder of the pre-mating exposure period (study weeks 1-10 for the 20 ppm group females and study weeks 3-10 for the 80 ppm group females). Mean food consumption values (measured as g/kg/day), in these females however, were also higher during this same exposure period when compared to the control group values. The differences were often statisticallysignificant (p<0.05 or p<0.01) and correlated with the lower mean body weights observed in these groups during study weeks 3-10. Correspondingly, food efficiency was reduced (p<0.01) in the 20 and 80 ppm males and in the 80 ppm females during study weeks 0-1 and in the 80 ppm males and females during study weeks 1-2. There were no other exposure-related effects on food efficiency. No exposure-related effects in mean food consumption, evaluated as g/animal/day and g/kg/day, and food efficiency were noted in the 5 ppm F0 male and female groups when compared to the control group. Slight differences were noted from control values but were not statistically significant and not considered related to test article exposure.
* GESTATION
Mean maternal food consumption, evaluated as g/animal/day and g/kg/day, and food efficiency in the 5, 20 and 80 ppm group F0 females were unaffected by test article exposure during gestation. The only statistically significant (p<0.05 or p<0.01) differences from the control group were increased mean food consumption, evaluated as g/kg/day, in the 20 and 80 ppm groups during gestation days 14-17 and decreased mean food consumption, evaluated as g/animal/day, in the 80 ppm group during gestation days 7-11. These slight increases and decreases were considered insignificant or were correlated to slightly lower mean body weights observed in these females during this period.
* LACTATION
Mean maternal food consumption, evaluated as g/animal/day and g/kg/day, and food efficiency in the 5, 20 and 80 ppm group F0 females were unaffected by test article exposure during lactation. Differences from the control group were slight and not statistically or biologically significant.

- REPRODUCTIVE PERFORMANCE, GESTATION LENGTH AND PARTURITION
No exposure-related effects on F0 reproductive performance were observed at any concentration. Male and female mating indices were 100.0%, 100.0%, 100.0% and 93.3% in the control, 5, 20 and 80 ppm groups, respectively. Male and female fertility indices were 96.7%, 93.3%, 96.7% and 90.0% and male copulation and female conception indices were 96.7%, 93.3%, 96.7% and 96.4% for the same respective groups. No statistically significant differences were noted between the control and test article-exposed groups. Males that did not sire a litter numbered 1, 2, 1 and 3 in the control, 5, 20 and 80 ppm groups, respectively. Females that had evidence of mating but did not deliver numbered 1, 2, 1 and 1 in the same respective groups. The mean numbers of days between pairing and coitus in the test article-exposed groups were similar to the control group value. No test article-related effects on F0 mean gestation length were observed at any exposure level. Mean gestation lengths in the control, 5, 20, and 80 ppm groups were 22.0, 21.8, 21.9, and 21.9 days, respectively.
- REPRODUCTIVE FUNCTION: ESTROUS CYCLE
The mean lengths of estrous cycles in the 5, 20 and 80 ppm group were also similar to the control group value. None of these differences werestatistically significant.
- REPRODUCTIVE FUNCTION: SPERM MEASURES
No exposure-related effects were observed in F0 spermatogenesis endpoints (mean testicular and epididymal sperm numbers and sperm production rate, motility, progressive motility and morphology) in males at any dosage concentration. Differences from the control group were slight and were not statistically significant.
- ORGAN WEIGHTSThe organ weight changes listed in TABLE 1 were statistically significant ($p<0.05$ or $p<0.01$) when compared to the control group but were generally considered to be a result of exposure-related effect on final body weights, which were 14% lower in males and 7.4% lower in females at 80 ppm compared to the control group. Changes not attributed to exposure-related effects on final body weights are discussed below.There was some variation in the degree of the organ weight changes. As a result of this variation, the lower absolute weight of the left epididymis and the higher left epididymis relative to body weight achieved statistical significance ($p<0.05$), while the lower absolute weight of the right epididymis did not reach a level of statistical significance and the higher right epididymis relative to body weight reached statistical significance ($p<0.01$). These organ weight changes were not considered biologically relevant in the absence of corresponding microscopic changes, changes in spermatogenic endpoints or alterations in functional reproductive outcome.Adrenal gland weight of females from the 80 ppm group was higher than control values when expressed as relative to body weight or brain weight. The effect on adrenal gland weight relative to body weight is intuitively apparent, as the body weight was lower and the adrenal gland weight remained constant. The basis for the effect on adrenal gland weight relative to brain weight is less obvious, as both adrenal gland and brain weight are expected to remain relatively constant despite lower body weight. Analysis of adrenal gland weight changes is confounded by additional factors, some of which are unknown or only crudely estimated in standard toxicology studies. For example, circumstances that result in loss of body weight, or failure to gain body weight, may involve prolonged, low-level stress to the animal and cause a systemic stress response, which commonly includes an increase in circulating corticosteroid levels and hypertrophy of adrenocortical cells. In this scenario the body weight is decreasing and the adrenal gland weight, which is predisposed to remain constant, is stimulated to increase due to a demand for corticosteroid hormone production. To further complicate interpretation of adrenal gland weights, circumstances that are acutely stressful to the animal may result in a massive release of pre-formed corticosteroid hormones from the adrenal cortex and an associated reduction in the size of adrenocortical cells (commonly called ‘adrenal exhaustion’). As a result of these interactions, interpretation of adrenal gland weights must be approached with great caution.Review of the organ weight data from the F0 generation animals reveals the absolute brain weights of males and females from the 80 ppm group were slightly lower than control values, but the difference did not achieve statistical significance. Absolute adrenal gland weights of females from the 80 ppm group were higher than control values, but the difference did not achieve a level of statistical significance. The combined effect of the slightly lower brain weight and higher adrenal gland weight resulted in a statistically significant difference in adrenal gland relative to brain weight of femalesfrom the 80 ppm group. The possibility of a stress response with associated adrenocortical alterations is supported by the histologic observation of increased cytoplasmic vacuolization in the adrenal cortex
- GROSS PATHOLOGYThere were no exposure-related macroscopic changes noted at the scheduled necropsy.
- HISTOPATHOLOGYAlthough cytoplasmic vacuolization was noted in the adrenal cortex of males from all exposure levels, including the controls, there was an increased incidence of adrenocortical cytoplasmic vacuolization in males from the 80 ppm group. Histopathologic examination of the adrenal gland of males from the 5 and 20 ppm groups revealed no alteration in the incidence of cytoplasmic vacuolization in the adrenal cortex. The increased level of cytoplasmic vacuolization of the adrenal cortex may be related to the altered adrenal gland organ weights, as discussed above. However, the microscopic changes in the adrenal cortex of the 80 ppm males were not observed in the F1 generation males. Therefore, a definitive resolution of these issues is beyond the scope of the present study.Other microscopic changes were considered to be incidental findings, manifestations of spontaneous diseases, or related to some aspect of experimental manipulation other than exposure to the test article. There was no exposure-related alteration in the incidence, severity or histologic character of those incidental and spontaneous tissue alterations. Evaluation of ovarian primordial follicles (10 sections from 10 randomly selected females were evaluated) showed no

evidence of an effect on the amount of primordial follicles. The mean number of ovarian primordial follicle counts in the F0 80 ppm group (61.9) was comparable to the control group value (60.9).
F1 GENERATION
- CLINICAL SIGNS AND MORTALITY
No test article-related deaths occurred in the F1 generation.No exposure-related clinical findings were noted during the F1 generation at the weekly examinations or at the midpoint exposure and 1-hour following exposure observations.
- BODY WEIGHT
* WEEKLY
Exposure-related decreased mean body weight gains (statistically significant, p<0.01) were noted in the 5, 20 and 80 ppm male groups and in the 20 and 80 ppm female groupsduring study week 18-19 and in the 5, 20 and 80 ppm males during study week 19-20. Decreased weekly mean body weight gains in the 20 and 80 ppm males continued throughout the remaining pre-mating and mating periods when compared to the control group; differences from the control were occasionally statistically significant (p<0.05 or p<0.01). Mean body weight gains in the 20 and 80 ppm female groups during this same interval were similar to the control group. As a result of lower gains during study week 18-19, 19-20 and/or the remaining pre-mating period, cumulative mean body weight changes in the 20 and 80 ppm male and 80 ppm female groups were statistically significantly (p<0.01) reduced during the pre-mating period (study weeks 18-30) when compared to the control group. Mean body weight gain in the 5 ppm male group was similar to the control group from study week 20 throughout the remaining treatment period. Cumulative mean body weight gains in this group were similar to the control group values during the pre-mating period (study weeks 18-30) and during the entire generation (study weeks 18-39). The slight, transient differences in male body weight gain from control values at 5 ppm were not considered biologically significant. Mean body weight gain in the 5 ppm female group was unaffected by test article exposure during the entire pre-mating and mating periods.Mean body weights in the 5, 20 and 80 ppm male groups were statistically significantly (p<0.05 or p<0.01; 19%, 14% and 17%, respectively, at study week 18) lower starting at the onset of exposure and continuing throughout the entire exposure period. Mean body weights in the 5, 20 and 80 ppm females were statistically significantly (p<0.05 or p<0.01; 13.5%, 12.4% and 15.7% at study week 18) lower at the onset of exposure when compared to the control group. Statistically significantly (p<0.01) lower mean body weights in the 80 ppm females continued to be observed throughout the remainingpre-mating and post-mating exposure periods when compared to the control group. During this same interim, lower mean body weights were noted (occasionally statistically significant, p<0.05 or p<0.01) in the 20 ppm females and mean body weights in the 5 ppm females were similar when compared to the control group. The decrements in mean body weights were attributed to test article exposure.
* GESTATION
Mean F1 maternal body weight gains in the 80 ppm group were statistically significantly decreased (p<0.05) when the entire gestation period (days 0-20) was evaluated as a result of lower (p<0.01) mean body weight gain during gestation days 17-20 compared to the control group. Mean body weights in this same group were 13.2% to 15.1% lower than the control group values throughout gestation. The differences from the control group values were statistically significant (p<0.01). These mean body weight decreases noted during gestation were a continuation of the decreases observed in this group during the pre-mating period.Mean maternal body weights, body weight gains and cumulative body weight changes in the 5 and 20 ppm groups were unaffected by test article administration. Increased (statistically significant, p<0.05) mean body weight gains in the 5 and 20 ppm groups were noted during gestation days 14-17. The increases did not occur in an exposure-related manner and were not considered test article-related.
* LACTATION
A statistically significantly (p<0.01) increased mean body weight gain was observed in the 80 ppm group during lactation days 1-4 when compared to the control group and was considered related to cessation of inhalation exposure during this time. The increase during lactation days 1-4 caused the overall lactation period (lactation days 1-28) to be increased (statistically significant, p<0.01) when compared to the control group. Statistically significantly (p<0.01) lower mean body weight was also noted in the 80 ppm F1 female group during the entire lactation period when compared to the control group. This difference from the control group was attributed to the lower mean body weight gains during the pre-mating period.Mean body weights and mean body weight gains in the 5 and 20 ppm groups were unaffected by test article exposure. Differences from the control group were slight and not statistically significant.
- FOOD CONSUMPTION
* WEEKLYMean food consumption, evaluated as g/animal/day, was statistically significantly (p<0.05 or p<0.01) reduced in the 5, 20 and 80 ppm males and in the 20 and 80 ppm females starting during study weeks 18-19 and 19-20. This trend continued throughout the remaining pre-mating period (study weeks 20-30) in the 20 and 80 ppm males and in the 80 ppm females. The differences from control were generally statistically significant (p<0.05 or p<0.01). Food consumption, evaluated as g/kg/day, in 5, 20 and 80 ppm male groups was increased starting at study weeks 20-21 and continuing throughout the entire pre-mating period when compared to the control group. The majority of the differences from control was statistically significant (p<0.05 or p<0.01). Food consumption, evaluated as g/kg/day, in the 20 and 80 ppm female groups was slightly increased (occasionally statistically significant, p<0.05 or p<0.01) starting on study weeks 20-21 and continuing throughout most of the pre-mating period when compared to the control.Decrements in

mean body weights in these animals correlated with these changes in food consumption and indicated that the males appeared to be more sensitive to exposure-related effects than the females.Statistically significantly (p<0.05 or p<0.01) increased food consumption (g/kg/day) was observed in the 5 ppm females starting on study weeks 18 to 26 and continuing (not statistically significant) throughout the remaining pre-mating period. This finding suggested a compensatory effect that correlated with slightly decreased mean body weight gain and slightly lower mean body weight noted at the initiation of direct exposure followed by normal mean body weights by the third week of exposure. Also correlating to lower mean body weights at the onset of exposure, food efficiency inthe 80 ppm males and females was statistically significantly (p<0.05) lower during study weeks 18-19 for males and females when compared to the control group. Following the initiation of exposure, food efficiency in all test article-exposed groups was generally similar or only slightly lower throughout the remaining pre-mating exposure when compared to the control group.
* GESTATION
Food consumption, evaluated as g/animal/day, was statistically significantly reduced in the 80 ppm group during gestation days 4-20 when compared to the control group. A corresponding statistically significant decrease in mean body weight gain was noted in this group during gestation days 0-20. Food consumption, evaluated as g/kg/day, and food efficiency in the 5, 20 and 80 ppm groups were unaffected by test article exposure.
* LACTATION
Food consumption, evaluated as g/animal/day and g/kg/day, and food efficiency in the 20 and 80 ppm groups were increased during lactation days 1-4; the differences were often statistically significant (p<0.05 or p<0.01) and were attributed to a compensatory effect due to the cessation of test article exposure. Food consumption (g/kg/day) and food efficiency in the 80 ppm group were also increased (p<0.05 or p<0.01) during evaluation of the entire lactation period, days 1-28, when compared to the control group. These findings corresponded with the compensatory higher body weight gain noted in the 80 ppm group during the cessation of test article exposure from lactation days 1-4 and the overall reduced mean body weights in the 20 and 80 ppm animals noted during pre-mating. No differences in food consumption in the 5 ppm group were noted when compared to the control group.
- REPRODUCTIVE PERFORMANCE
No exposure-related effects on F1 reproductive performance were observed at any concentration. Male and female mating indices were 93.3%, 89.7%, 100.0% and 93.3% in the control, 5, 20 and 80 ppm groups, respectively. Male and female fertility indices were 83.3%, 86.2%, 93.1% and 90.0%, and male copulation and female conception indices were 89.3%, 96.2%, 93.1% and 96.4% for the same respective groups. No statistically significant differences were noted between the control and test article-exposed groups. Males that did not sire a litter numbered 5, 4, 2 and 3 in the control, 5, 20 and 80 ppm groups, respectively. The numbers of females that had evidence of mating but did not deliver were 3, 1, 2 and 1 in the same respective groups.The mean numbers of days between pairing and coitus in the test article-exposed groups were similar to the control group value. No exposure-related effects were noted on mean gestation lengths or the process of parturition at any concentration. Mean F1 gestation lengths in the control, 5, 20 and 80 ppm groups were 22.1, 21.8, 21.7 and 22.1 days.
- REPRODUCTIVE FUNCTION: ESTROUS CYCLE
The mean lengths of estrous cycles in the 5, 20 and 80 ppm groups were also similar to the control group value. None of these differences were statistically significant.- REPRODUCTIVE FUNCTION: SPERM MEASURES No exposure-related effects were observed on F1 spermatogenesis endpoints (mean testicular and epididymal sperm numbers and sperm production rate, motility, progressive motility and morphology) in males at any dosage concentration.
- ORGAN WEIGHTS
The organ weight changes lisyed in TABLE 2 were statistically significant when compared to the control group but were generally considered to be a result of exposure-related effect on final body weights. While the lower body weight achieved a level of statistical significance only in males from the 20 and 80 ppm exposure levels and females from the 80 ppm exposure level, final body weight of males from the 5 ppm and females from the 20 ppm groups were also lower than control values. The lower final body weight of 5 ppm males and 20 ppm females influenced the analysis of a number oforgan weights, resulting in statistically significant alterations in some organ weights when expressed relative to final body weight.The organ weight data in the F1 animals indicate, as expected, that adrenal gland and brain weights did not decline in synchrony with body weights. Brain weights of test article-exposed animals were only slightly lower than control values, but the slight difference achieved statistical significance for 5 and 80 ppm males and 80 ppm females. Absolute adrenal gland weights of test article-exposed males remained relatively constant. Adrenal gland weights of test article-exposed females remained relatively constant or, in the case of 80 ppm females, were higher than control values. The final result was that adrenal gland weights of males and females exposed at the 80 ppm level were significantly higher than control values when expressed relative to final body weight or brain weight. This apparent adrenal weight alteration was attributed either directly or indirectly to the test article-related effect on final body weight, with an unknown contribution due to generalized stress and a stress response.There were no other exposure-related effects on organ weights. A difference in absolute left testis weight at 5 ppm was statistically significant when compared to the control group, but the absolute weight and weights relative to body and brain weight were discordant and were not observed in an exposure-related manner; thus the changes were considered to be spurious.
- GROSS PATHOLOGY (PARENTAL ANIMALS)

There were no exposure-related macroscopic changes noted at the scheduled necropsy.
- HISTOPATHOLOGY (PARENTAL ANIMALS)
There were no exposure-related microscopic changes. A mammary adenocarcinoma was identified in 1 of 30 females from the F1 generation 20 ppm group. Although such a finding is unusual in an animal of that age, it was considered incidental based on its single occurrence and the lack of an exposure-response relationship. Evaluation of ovarian primordial follicle counts (10 sections from 10 randomly selected females were evaluated) showed no evidence of an effect on the amount of primordial follicles. The mean number of ovarian primordial follicle counts in the F1 80 ppm group (77.9) was comparable to the control group value (61.5).

Results of examinations: offspring

Viability (offspring) : no effects

Clinical signs (offspring) : no effects

Body weight (offspring) : no effects

Sexual maturation (offspring) : no effects

Organ weights (offspring) : no effects

Gross pathology (offspring) : no effects

Histopathology (offspring) : not examined

Details on results (offspring)

F1 GENERATION- PND 0 LITTER DATA AND POSTNATAL SURVIVAL
The mean number of pups born, live litter size, percentage of males per litter at birth and postnatal survival between birth and PND 0 (relative to number born), PND 0-1, 1-4 (pre-selection), 4 (post-selection)-7, 7-14, 14-21, and from birth to PND 4 (pre-selection) and PND 4 (post-selection)-28 were unaffected by the test article at all exposure concentrations. Differences from the control group were slight, not statistically significant and did not occur in an exposure-related manner.
- GENERAL PHYSICAL CONDITION AND MORTALITIES
The numbers of F1 pups found dead and/or missing, as well as the general physical condition of all F1 pups in this study were unaffected by parental test article exposure.
- OFFSPRING BODY WEIGHTS (TABLE 3)
Mean F1 male and female pup body weight and body weight gains in the 5, 20 and 80 ppm groups were similar to the control group during the period of suspended F0 maternal exposure (PND 1-4). Mean F1 male and female pup body weight gains in the 5 ppm group were decreased when compared to the control group during PND 4-7. However, there was no exposure-related relationship, and mean body weights in these pups were only slightly lower than those of the concurrent control group on PND 4 and 7. Mean male and female pup body weight gains in the 5, 20 and 80 ppm groups were slightly lower during PND 7-14, 14-21 and 21-28 when compared to the control group, but no exposure-related response was evident. Overall, statistically significant decreases in mean body weight gains were noted in the male and female pups in the 5, 20 and 80 ppm groups from PND 4 to 28. Mean body weights were statistically significantly decreased in the 5 ppm males on PND 14, in the 5 and 20 ppm males and 5 ppm females on PND 21 and in the 5, 20 and 80 ppm males and 5 and 80 ppm females on PND 28 when compared to the control group. By PND 28, mean male pup body weights in the 5, 20 and 80 ppm groups were 12.0%, 8.7% and 9.7% lower, respectively, than the control group, and mean female pup body weights were 9.8%, 7.3% and 9.1% lower in the same respective groups. However, there was no exposure-response relationship noted between the test article-exposed groups, and the 5 ppm group had the lightest body weights and the lowest overall weight gains.Comparison of the F1 pup data with historical control values was confounded by the fact that the PND 4 pups in the test article-exposed groups had mean body weights slightly above the mean values in the WIL historical control data for inhalation studies, while the PND 28 values in these groups were slightly lower (7% or less) than the mean values in the historical control data. In addition, mean body weights in the concurrent control group were notably higher (4.2% to 5.3%) when compared to the mean values in the WIL historical control data for inhalation studies on PND 28.
- NECROPSIES OF PUPS FOUND DEAD OR EUTHANIZED IN EXTREMIS
The numbers of pups (litters) found dead or euthanized in extremis during PND 0-28 numbered 12(11), 16(12), 40(11) and 14(8) in the control, 5, 20 and 80 ppm groups, respectively. No internal findings that could be attributed to F0 parental exposure to the test article were noted in any group.
-NECROPSIES OF WEANLINGS NOT SELECTED FOR ORGAN WEIGHTS
- PND 28: The numbers of pups (litters) examined viscerally on PND 28 numbered 106(29), 103(28), 102(27) and 99(27) in the control, 5, 20 and 80 ppm groups, respectively. No internal findings in any group were noted that could be

attributed to F0 parental exposure to the test article.
- NECROPSIES OF WEANLINGS SELECTED FOR ORGAN WEIGHTS
- PND 28
* MACROSCOPIC EXAMINATIONAt the PND 28 necropsy of F1 weanlings selected for organ weights, internal findings included kidney cyst in a pup in the control group, adhesion on spleen (attached to the left lateral abdominal wall) in a pup in the 5 ppm group and dark red discoloration of the mandibular salivary gland in a pup and a small thymus in a pup in the 80 ppm group. These isolated findings were not attributed to test article exposure.
* ORGAN WEIGHTS No effects on mean organ weights (absolute, relative to final body weight and relative to brain weight) were observed at any dosage level when the test article-exposed groups were compared to the control group.
- F1 DEVELOPMENTAL LANDMARKS
* BALANOPREPUTIAL SEPARATION
Mean ages of attainment of balanopreputial separation were 47.7, 45.8 and 46.7 days in the 5, 20 and 80 ppm groups, respectively, compared to 44.9 days in the concurrent control group; the difference from control was statistically significant in the 5 ppm group and no exposure-response was observed. The mean value for the age of attainment of balanopreputial separation age in the WIL historical control data for inhalation studies was 46.3 days, suggesting that the differences observed in the 5 ppm group was not biologically significant and unrelated to test article exposure.
* VAGINAL PATENCYMean age at attainment of vaginal patency in the 5, 20 and 80 ppm groups was 36.2, 35.9 and 36.4 days, respectively, compared to 34.4 days in the concurrent control group. The differences in these same respective groups were statistically significant ($p<0.05$ or $p<0.01$) when compared to the concurrent control group; however no exposure-response relationship was observed. The mean value in the WIL historical control data for inhalation studies was 35.2 days with a range of 32.5 to 38.8 days and indicated that the differences noted in this study were unrelated to test article exposure.
F2 GENERATION
- PND 0 LITTER DATA AND POSTNATAL SURVIVALThe mean live litter size, percentage of males per litter at birth and postnatal survival between birth and PND 0 (relative to number born), PND 0-1, PND 1-4 (pre-selection), PND 4-7 (post-selection), PND 7-14, PND 14-21, PND 21-28, birth to PND 4 (preselection), and PND 4 (post-selection)-28 were unaffected by the test article at all exposure levels.A female in the control group and a female in the 80 ppm group had total litter losses between PND 0-2.
- GENERAL PHYSICAL CONDITION AND MORTALITIESThe numbers of F2 pups found dead and/or missing, as well as the general physical condition of all F2 pups in this study were unaffected by F1 parental test article exposure.
- OFFSPRING BODY WEIGHTSMean F2 pup body weight gain in test article-exposed groups was similar or slightly lower during the period of suspended F1 maternal exposure (PND 1-4) and following re-initiation of F1 maternal exposure (PND 4-7, 7-14, 14-21, 21-28 and 4-28) when compared to the concurrent control group values. Minor differences from control were not statistically significant (during PND 1-4 and 4-28), did not demonstrate an exposure-related relationship and were considered related to differences in live litter size between the control and test article-exposed groups. Mean F2 pup body weights were also similar or slightly lower (not statistically significant) during the entire lactation period when compared to the concurrent control group. The mean body weight values in the male and female concurrent control group were higher than the mean values in the WIL historical control data for inhalation studies at PND 1 (2.8% and 4.5%, respectively) and at PND 28 (5.3% and 7.0%, respectively). Therefore, slight differences noted in the test article-exposed groups compared to the concurrent control values were attributed to higher mean values in the concurrent control and were not considered biologicallysignificant. In conclusion, mean pup body weight gains and body weights were unaffected at any exposure level throughout the lactation period.
- NECROPSIES OF PUPS FOUND DEADThe numbers of pups (litters) found dead during PND 0 through the selection of the F2 generation numbered 9(7), 14(7), 9(7) and 13(8) in the control, 5, 20 and 80 ppm groups, respectively. No internal findings that could be attributed to parental exposure to the test article were noted at the necropsies of pups that were found dead.
- NECROPSIES OF WEANLINGS NOT SELECTED FOR ORGAN WEIGHTS
- PND 28: At the PND 28 necropsy, no internal findings that could be attributed to F1 maternal test article exposure were noted at the necropsy of pups euthanized on PND 28.
- NECROPSIES OF WEANLINGS SELECTED FOR ORGAN WEIGHTS
- PND 28
* MACROSCOPIC EXAMINATIONAt the PND 28 necropsy of F2 weanlings selected for organ weights, internal findings included cyst(s) on right kidneys in 1 pup in each of the control and 5 ppm groups, dilated pelvis in the kidney in 1 pup in each of the 5 and 80 ppm groups and dark red areas in the thymus in 1 pup in each of the control, 5 ppm and 80 ppm groups. These findings were not considered test article-related because they occurred randomly in single pups.
* ORGAN WEIGHTS (OFFSPRING)No test article-related effects on organ weights (absolute, relative to final body weight and relative to brain weight) were observed at any dosage concentration when the test article-exposed groups were compared to the control group.

Any other information on results incl. tables

Text Table 1: Organ weight changes associated with body weight changes at 80 ppm in F0generation animals

Organ	Direction of change[a]	Sex
Brain (relative to body weight)	increase	Male, Female
Cauda, epididymis, right (relative to body weight)	increase	Male
Epididymis, left (absolute)	-	Male
Epididymis, left (relative to body weight)	increase	Male
Epididymis, right (relative to body weight)	increase	Male
Testis, left (relative to body weight)	increase	Male
Testis, right (relative to body weight)	increase	Male
Kidneys (absolute)	decrease	Male
Kidneys (relative to brain weight)	decrease	Male
Liver (absolute)	decrease	Male
Liver (relative to brain weight)	decrease	Male
Spleen (absolute)	decrease	Male
Spleen (relative to brain weight)	decrease	Male
Adrenal glands (relative to body weight)	increase	Female
Adrenal glands (relative to brain weight)	increase	Female
Final Body Weight[b]	decrease	Male, Female

a= The indication of higher or lower refers to a statistically significant difference between test article-exposed and control group animals.

b= Final body weights in the 80 ppm males and females (491 g and 324 g, respectively) were significantly different from the control group (571 g and 350 g for males and females, respectively) at 0.01 using Dunnett's test.

Text Table 2 : Organ weight changes associated with body weight changes in F1generation animals

Organ	Direction of change(a	Sex	Exposure level (ppm)
Brain (absolute)	decrease	Female Male	80 20, 80
Brain (relative to body weight)	increase	Female	80
Cauda, epididymis, left (absolute)	decrease	Male	80
Cauda, epididymis, left (relative to body weight)	increase	Male	20
Cauda, epididymis, right (relative to bodyweight)	increase	Male	20, 80
Epididymis, left (relative to body weight)	increase	Male	20, 80
Epididymis, right (absolute)	decrease	Male	80
Epididymis, right (relative to body weight)	increase	Male	20, 80
Testis, left (absolute)	decrease	Male	80
Testis, left (relative to body weight)	increase	Male	80
Testis, right (absolute)	decrease	Male	5, 20, 80
Testis, right (relative to body weight)	increase	Male	80
Kidneys (absolute)	decrease	Male Female	20, 80 80
Kidneys (relative to brain weight)	decrease	Male, Female	80
Liver (absolute)	decrease	Male Female	20, 80 80
Liver (relative to brain weight)	decrease	Male, Female	80
Spleen (absolute)	decrease	Male, Female	80
Spleen (relative to brain weight)	decrease	Male	80
Adrenal glands (relative to body weight)	increase	Male, Female	80
Adrenal glands (relative to brain weight)	increase	Female	80
Pituitary (absolute)	decrease	Male	5, 20, 80
Pituitary (relative to brain weight	decrease	Male	5, 20, 80
Prostate (relative to body weight)	increase	Male	80
Seminal vesicles/coagulating glands/fluid (absolute)	decrease	Male	80
Final Body Weight (b	decrease decrease	Male Female	5, 20, 80 20, 80

a= The indication of higher or lower refers to a statistically significant difference between test article-exposed and

control group animals.
b= Final body weights in the males in the 20 and 80 ppm groups (549 g and 522 g, respectively) and in the females in the 80 ppm group (294 g) were significantly different from the control group (614 g and 340 g for males and females, respectively) at 0.01 using Dunnett’s test. Final body weights in the males in the 5 ppm group and females in the 20 ppm group (574 g and 329 g, respectively) were not statistically different.

TABLE 3: F1 - PRE-WEANING - SUMMARY OF OFFSPRING WEIGHTS [G] (LITTER AS EXPERIMENTAL UNIT)

GROUP:	SEX		0 PPM	5 PPM	20 PPM	80 PPM
PND 1	MALES	MEAN	7.2	7.1	7.1	7.1
		S.D.	0.73	0.71	0.78	0.60
		N	29	28	29	27
	FEMALES	MEAN	6.7	6.7	6.7	6.7
		S.D.	0.58	0.76	0.66	0.54
		N	29	28	29	27
PND 4 (BEFORE SELECTION)	MALES	MEAN	10.1	9.8	10.2	10.1
		S.D.	1.47	1.37	1.27	1.11
		N	29	28	28	27
	FEMALES	MEAN	9.3	9.4	9.6	9.6
		S.D.	1.26	1.42	1.13	1.05
		N	29	28	28	27
PND 7	MALES	MEAN	13.8	12.7	13.3	13.5
		S.D.	2.28	1.80	1.96	1.97
		N	29	28	28	27
	FEMALES	MEAN	12.8	12.1	12.6	12.7
		S.D.	2.18	2.02	1.82	1.87
		N	29	28	28	27
PND 14	MALES	MEAN	26.4	23.8*	24.5	24.7
		S.D.	3.69	3.71	3.65	2.75
		N	29	28	28	27
	FEMALES	MEAN	24.9	22.9	23.5	23.4
		S.D.	3.92	3.94	3.49	2.48
		N	29	28	28	27
PND 21	MALES	MEAN	42.0	36.4**	38.2*	37.9
		S.D.	6.47	6.66	6.30	5.79
		N	29	28	28	27
	FEMALES	MEAN	39.6	35.1*	36.7	36.2
		S.D.	6.10	6.50	6.15	5.28
		N	29	28	28	27
PND 28	MALES	MEAN	79.7	70.1**	72.8*	72.0*
		S.D.	10.	66 11.	70 10.	94 9.88
		N	29	28	28	27
	FEMALES	MEAN	72.7	65.6*	67.4	66.1*
		S.D.	9.99	10.58	9.	67 8.36
		N	29	28	28	27

PND = POSTNATAL DAY
* = SIGNIFICANTLY DIFFERENT FROM THE CONTROL GROUP AT 0.05 USING COVARIATE ANALYSIS
** = SIGNIFICANTLY DIFFERENT FROM THE CONTROL GROUP AT 0.01 USING COVARIATE ANALYSIS

Applicant's summary and conclusion

Conclusions

In a reproduction study, four groups of male and female Crl:CD(SD) rats (30/sex/group) were exposed to either clean filtered air or vapor atmospheres of DMDS for 6 hours daily over 2 generations The exposure concentrations were 0, 5, 20 and 80ppm. The first parental generation (F0) was exposed for at least 70 consecutive days prior to mating. General systemic toxicity was seen in the 20 and 80 ppm F0 and F1 parental males and females characterized by decreased mean body weights. body weight gains and/or food consumption. The NOAEL for parental systemic toxicity was 5

ppm. No functional effects on reproduction or on pups were seen at any tested concentration. The NOAEL for reproductive and developmental toxicity was 80 ppm.

Executive summary

In a 2 -generation reprotoxicity study performed following the OECD guideline # 416, four groups of male and female Crl:CD) SD) rats (30/sex/group) were exposed to either clean filtered air or vapor atmospheres of the test article, dimethyl disulfide, for 6 hours daily for at least 70 consecutive days prior to mating at DMDS concentrations of 0, 5, 20 and 80 ppm for the F0 and F1 generations. The first parental generation F0 animals were approximately 6.6 weeks of age at the beginning of exposure. The offspring selected to become the F1 parental generation were exposed following weaning (beginning on postnatal day [PND] 28). Exposure of the F0 and F1 males continued throughout mating and through the day prior to euthanasia. The F0 and F1 females continued to be exposed throughout mating and gestation through gestation day 20. After parturition, exposure of the F0 and F1 females was re-initiated on lactation day 5 and continued through the day prior to euthanasia. F0 animals were exposed for 142 consecutive days, and F1 animals were exposed for 158 consecutive days. All animals were observed twice daily for appearance and behavior. Clinical observations, body weights and food consumption weree recorded at appropriate intervals for males throughout the study and for females prior to mating and during gestation and lactation. Vaginal lavage was performed daily to determine estrous cycles beginning 21 days prior to pairing. All F0 and F1 females were allowed to deliver and rear their pups until weaning on lactation day 28. Clinical observations, body weights and sexes for F1 and F2 pups were recorded at appropriate intervals. For both generations (F1 and F2), 8 pups per litter (4 per sex, when possible) were selected on PND 4 to reduce the variability among the litters. Offspring (30/sex/goup) from the pairing of the F0 animals were selected by PND 21 to constitute the F1 generation. Developmental landmarks (balanopreputial separation and vaginal patency) were evaluated for the selected F1 rats. Nonseleeted F1 and F2 weanlings were necropsied on PND 28. Selected organs were weighed from both F1 and F2 pups that were necropsied on PND 28. Each surviving F0 and F1 parental animal received a complete detailed gross necropsy following the completion of weaning of the F1 and F2 pups, respectively; selected organs were weighted. Spermatogenic endpoints (sperm motility including progressive motility, morphology and numbers) were recorded for all surviving F0 and F1 males, and ovarian primordial follicle counts were recorded for all surviving F0 and F1 females, in the control and high-exposure groups. Designated tissues from all F0 and F1 parental animals were weighed and examined microscopically.
There were no functional effects on reproduction (estrous cycles, mating and fertility indices, number of days between pairing and coitus, and gestation length) in any test article-exposed group. There were no adverse effects on pups born to dams exposed to the test article and results from several studies confirm a lack of effect on postnatal growth prior to weaning with exposure of the lactating dams. Therefore, in this study an exposure level of 80 ppm was considered to be the no-observed-adverse-effect level (NOAEL) for reproductive and developmental toxicity when the test article was administered via whole-body inhalation exposure to Crl:CD(SD) rats. General systemic toxicity was evident in the 20 and 80 ppm group F0 and F1 parental males and females with persistent decrements in mean body weights, body weight gains and/or food consumption. Potential exposure-related effects on the adrenal glands (an increase in the incidence of vacuolization of the adrenal cortex or increased adrenal gland weights [relative to final body weight or brain weight]) were noted in the F0 and F1 parental animals in the 80 ppm group. The F0 and F1 5 ppm parental groups were not affected by these parameters other than slight transitory decreases in body weight gain and/or food consumption. Therefore, a NOAEL for parental systemic toxicity was considered to be 5 ppm.

[Study 2] Nemec (WIL) 2006/K1 SS/Reproduction/developmental toxicity screening assay

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Supporting Stduy	1 (reliable without restriction)	Nemec MD	2006	A reproduction/developmental toxicity screening inhalation study of dimethyl disulfide in rats

Materials and methods

Test type : screening

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test)	no

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Purity: : 99.5% (certificate of analysis)
99.73% (beginning of the study)
99.71% (end of the study)

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Laboratories, Inc., Raleigh, North Carolina,
- Age at reception: 58 days old
- Body weight at exposure initiation: (P) Males: 342 - 392 g; Females: 219 - 274 g
- Housing (non exposure periods): individually in wire-mesh cages (except during the exposure period) suspended above cage-board.
- Diet : PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002, ad libitum. Food was withheld during the exposure periods.
- Water: municipal water, ad libitum. Water was withheld during the exposure periods.
- Acclimation period: 14 days

ENVIRONMENTAL CONDITIONS (NON-EXPOSURE PERIODS)
- Temperature (°C): 22 ± 3 (actual : 21.2°C to 21.7°C)
- Humidity (%): 50 ± 20 (actual: 36.8% to 47.8%)
- Air changes (per hr): 12
- Photoperiod (hrs dark / hrs light): 12/12

IN-LIFE DATES: From: 2005-02-22 To: 2005-06-14

Administration / exposure

Route of administration : inhalation: vapour

Type of inhalation exposure (if applicable) : whole body

Vehicle : unchanged (no vehicle)

Details on exposure

Exposures were conducted in four 1.0-m3 stainless steel and glass exposure chambers. One chamber was dedicated to each of the 3 test article exposure groups and the 4th chamber was dedicated to the control group. The exposure period was 6 hours per day, 7 days per week and was defined as the time between turning the generation system on and off, including the start equilibration time. The chambers were operated under dynamic conditions, at a slight negative pressure (ca 0.5 in of water) with at least 12 to 15 air changes per hour.

Details on mating procedure

The animals were paired on a 1:1 basis within each exposure group following 14 days of exposure for the males and females. A breeding record containing the male and female identification numbers and the start date of cohabitation was

prepared. Each female was housed in the home cage of the male. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm following a vaginal lavage. Each mating pair was examined daily. The day when evidence of mating was identified was termed gestation day 0. If evidence of copulation was not detected after 14 days of pairing, any females that had not shown evidence of mating were placed in plastic maternity cages.

Analytical verification of doses or concentrations : yes

Details on analytical verification of doses or concentrations

Actual exposure concentrations within each chamber were measured at least 10 times (approximately every 35 minutes) during each daily exposure period by a validated gas chromatographic method. Exposure atmosphere samples were delivered to the gas chromatograph from the approximate middle of each chamber by a pump and multi-port sampling valve. At least 1 standard was analyzed each day prior to exposure to confirm gas chromatographic calibration. Overall mean measured test article exposure concentrations for the F0 generation were 0, 5, 50 and 150 ppm and for the F1 generation were 0, 5.1, 50 and 150 ppm for the filtered air, 5, 50 and 150 ppm groups, respectively.

Duration of treatment / exposure

The F0 males and females were exposed to the test atmosphere for 14 consecutive days prior to mating. Exposure of the F0 males continued throughout mating and through the day prior to euthanasia. The F0 females continued to be exposed throughout mating through gestation day 20; exposure was re-initiated on lactation day 5 and continued through lactation day 27. During lactation, the dams were removed from their litters during each daily 6-hour exposure period. One F1 pup/sex/litter was selected for inhalation exposure beginning on PND 28 (following weaning) and continuing until PND 34.

Frequency of treatment : 6 hours per day, 7 days per week

Doses / concentrations : 5, 50 and 150 ppm

Basis analytical conc .

No. of animals per sex per dose : 12

Control animals : other: The control group was exposed to clean, filtered air under conditions identical to those used for the groups exposed to test article.

Positive control : Not appropriate

<u>Examinations</u>

Parental animals: Observations and examinations

CAGE SIDE OBSERVATIONS: Yes

All F0 rats were observed twice daily, once in the morning and once in the afternoon, for appearance, behavior, moribundity and mortality. During inhalation exposures, animals that were visible through the chamber windows were observed for appearance and behavior at the mid-point of exposure. In addition, each animal was observed for signs of toxicity within 1 hour following exposure. All significant findings were recorded.

DETAILED CLINICAL OBSERVATIONS: Yes

Individual detailed clinical observations were recorded weekly.

BODY WEIGHT: Yes

Individual male body weights were recorded on the first day of exposure, weekly thereafter and at termination.- Individual female body weights were recorded on the first day of exposure, weekly thereafter until evidence of copulation was observed. Once evidence of mating was observed, female body weights were recorded on gestation days 0, 4, 7, 11, 14, 17 and 20 and on lactation days 1, 4, 7, 14, 21 and 28.

FOOD CONSUMPTION :

Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes

WATER CONSUMPTION : No

Estrous cyclicity (Parental animals)

Not done.

Sperm parameters (Parental animals)

Not done

Litter observations

STANDARDISATION OF LITTERS
- Performed on day 4 postpartum: yes
- If yes, maximum of 10 pups/litter (5/sex/litter as nearly as possible); excess pups were killed and discarded.

F1 LITTER PARAMETERSLITTER VIABILITY AND DEATHS
Each litter was examined daily for survival, and all deaths were recorded.
CLINICAL OBSERVATIONS
Litters were examined daily for survival and any adverse changes in appearance or behavior. Each pup received a detailed physical examination on PND 1, 4, 7, 14, 21 and 28.
BODY WEIGHTS
Pups were individually weighed on PND 1, 4, 7, 14, 21 and 28.
SEX DETERMINATION
Pups were individually sexed on PND 0, 1, 4, 14 and 28.
WEANING AND SELECTION
Each dam and litter remained housed together until weaning on lactation day 28. One male and 1 female F1 weanling from each group (control, 5, 50 and 150 ppm) were randomly selected using a computerized randomization procedure prior to weaning (PND 28) to comprise the F1 generation. These pups (a minimum of 1 male and 1 female per litter, when available) were exposed to the control or test article for 6 hours per day beginning on day 28 post partum.

Postmortem examinations (Parental animals)

F0 MACROSCOPIC EXAMINATIONS
Males were euthanized following completion of the mating period. Females that delivered were euthanized on lactation day 28 and females with total litter loss were euthanized within 24 hours of litter loss; the numbers of former implantation sites were recorded.Necropsy included examination of the external surface, all orifices and the cranial cavity, the external surface of the brain, and the thoracic, abdominal and pelvic cavities, including viscera. Uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss.
ORGAN WEIGHTS
The following organs were weighed from all F0 animals at the scheduled necropsies:Brain OvariesEpidiymidesa PituitaryKidneys Testes(aLiver(a = These paired organs were weighed separately.
MICROSCOPIC EXAMINATIONS
Microscopic examination was performed on the following tissues from all animals in the control and 150 ppm groups at the scheduled necropsies and from the animal euthanized in extremis during the study.Cervix, Coagulating gland, Epididymides, Mammary glands, Ovaries (2), Pituitary gland, Prostate gland, Seminal vesicles (2), Testes, Vas deferens, Uterus, Vagina, All gross (internal) lesions

Postmortem examinations (Offspring)

EUTHANASIA OF F1 GENERATION
F1 pups not selected for exposure were euthanized via isoflurane inhalation on PND 28 and examined internally for gross abnormalities.F1weanlings selected for exposure were euthanized via carbon dioxide inhalation on PND 35 and discarded without macroscopic examination.

Statistics

Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test article-exposed group to the control group by sex. Where applicable, the litter was used as the experimental unit. Parental mating, fertility, conception and copulation indices were analyzed using the Chi-square test with Yates' correction factor. Mean parental body weights (weekly, gestation and lactation), body weight changes and food consumption, offspring body weights and body weight changes, gestation length,numbers of implantation sites, number of pups born, live litter size on PND 0, unaccounted-for sites, absolute and relative organ weights, pre-coital intervals values were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test article-exposed groups to the control group. Mean litter proportions (percent per litter) of males at birth and postnatal survival were subjected to the Kruskal-Wallis nonparametric ANOVA to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$)intergroup variance, Dunn's test (Dunn, 1964) was used to compare the test article-exposed groups to the control group. Mean litter proportions (percent per litter) of males

at birth and postnatal survival were subjected to the Kruskal-Wallis nonparametric ANOVA to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunn's test was used to compare the test article-exposed groups to the control group. Histopathological findings in the test article-exposed groups were compared to the control group using a two-tailed Fisher'sExact test.

Reproductive indices

Mating, fertility and copulation, conception indices were calculated.

Offspring viability indices

The following litter parameters were calculated: Mean Live Litter Size, Postnatal Survival Between Birth and PND 0 or PND 4, Postnatal Survival for All Other Intervals

Results and discussions

Effect levels

Endpoint	**Generation**	**Sex**	**Effect level**	**Based on**	**Basis for effect level / Remarks**
NOAEC (parental systemic toxicity)	P	male/female	5 ppm (analytical)		Decrements in body weight gain and food consumption in the 50 (males only) and 150 ppm groups.
NOAEC (parental reproductive toxicity)	P	male/female	150 ppm (analytical)		No functional effects on reproduction at any exposure concentration.
NOAEC (neonatal toxicity)	F1	male/female	5 ppm (analytical)		Reduced F1 pup body weights and body weight gains.

Results of examinations: parental animals

Clinical signs (parental animals) : no effects

Body weight and food consumption (parental animals) : yes

Reproductive function: estrous cycle (parental animals) : not examined

Reproductive function: sperm measures (parental animals) : not examined

Reproductive performance (parental animals) : no effects

Organ weights (parental animals) : no effects

Gross pathology (parental animals) : no effects

Histopathology (parental animals) : no effects

Details on results (parental animals) : CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS)
One F0 female in the 150 ppm group was euthanized in extremis on lactation day 7 following a body weight loss of 39 g. This female had several clinical findings prior death, including a pale body and eyes, shallow respiration and red material around the nose and mouth. A cause of death could not be determined at necropsy or microscopically (with a limited number of tissues being examined). However, the moribund condition of this female was considered test article-related since body weight effects were noted for other animals in this group and spontaneous mortality is low in rats, especially during lactation. All other animals survived to the scheduled necropsies. No test article-related clinical findings were observed in the F0 males and surviving F0 females.
BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS)
Effects on mean body weights, body weight changes and food consumption were noted in the 50 and 150 ppm group F0 males throughout the study and in the 150 ppm group F0 females during gestation and lactation. During the first week of the premating period (study week 0-1), a mean body weight loss was noted at 150 ppm, while a reduced mean body weight gain was noted at 50 ppm compared to the control group. Mean body weight gains in the 150 ppm group were reduced for the remainder of the study (study weeks 2-3 and 3-4) while mean body weight gains in the 50 ppm group were reduced during study week 3-4. As a result, mean body weights in the 50 and 150 ppm group F0 males were up to 5.5% and 12.6% less than the control group, respectively, during study weeks 1-4. Mean F0 maternal body weight gains were reduced during most of the gestation period, and during lactation days 4-7 (following resumption of test article exposure). Mean body weights in this group were up to 12.5% and 10.0% lower than the control group during the

gestation and lactation periods, respectively. Mean F0 maternal food consumption was generally reduced in the 150 ppm group females throughout gestation and lactation.There were no effects on body weights, body weight gains or food consumption in F0 males at 5 ppm and in F0 females at 5 and 50 ppm. Mean body weights in the 50 ppm group F0 females were lower on lactation days 1 and 7. The slight reductions (approximately 6%) were attributed to the reduced mean body weight gains noted during the premating period and were not considered evidence of toxicity during lactation.
REPRODUCTIVE PERFORMANCE (PARENTAL ANIMALS)
One F0 female in the 5 ppm group had total litter loss on lactation day 1. No total litter loss occurred at 50 or 150 ppm; therefore, the occurrenceat 5 ppm was not considered test article-related.Reproductive performance (mating, fertility, copulation and conception indices) was unaffected by exposure to the test article. The mean numbers of days between pairing and coitus and mean gestation lengths were unaffected by test article exposure. There were no signs of dystocia in this study.A slight reduction in the mean number of implantation sites in the 150 ppm group was attributed to a single female with an atypically low value (4 implantation sites); no relationship to the test article was evident. The mean number of implantation sites in the 5 and 50 ppm groups and the mean number of unaccounted for implantation sites in the 5, 50 and 150 ppm groups were similar to the control group value.
ORGAN WEIGHTS (PARENTAL ANIMALS)
No test article-related effects on organ weights were noted in the F0 males or females. Any differences from the control group were slight, did not occur in an exposure-related manner, or were explained by reduced mean body weights.
GROSS PATHOLOGY (PARENTAL ANIMALS)
There were no test article-related macroscopic findings in F0 males andfemales.
HISTOPATHOLOGY (PARENTAL ANIMALS)
There were no test article-related microscopic findings in F0 males andfemales.

Results of examinations: offspring

Viability (offspring) : no effects

Clinical signs (offspring) : no effects

Body weight (offspring) : yes

Sexual maturation (offspring) : not examined

Organ weights (offspring) : not examined

Gross pathology (offspring) : not examined

Histopathology (offspring) : not examined

Details on results (offspring)

VIABILITY (OFFSPRING)
The mean number of pups born and live litter size on PND 0 were slightly lower in the 150 ppm group as a result of a single female, which also had an atypically low number of implantation sites. No relationship to the test article was evident. No test article-related effects on the mean number of pups born, live litter size and the percentage of males at birth were observed in the 5 and 50 ppm groups. Postnatal survival in the 150 ppm group was lower during PND 4-28 due to a single female that lost 5 pups during PND 5-9. There were no effects of maternal exposure to the test article on postnatal survival in the 5 and 50 ppm groups.
CLINICAL SIGNS (OFFSPRING)
The general physical condition of the F1 pups was not affected by maternal exposure to the test article. There were no clinical findings in the F1 pups selected for 1 week of direct test article exposure during the post-weaning period.
BODY WEIGHT (OFFSPRING)
Mean F1 male and femalepup body weight gains were lower in the 50 and 150 ppm groups during PND 4-28 (females) and PND 7-14 (males). As a result, mean body weights in the 50 and 150 ppm groups were up to 15.3% and 17.0% less (females) and up to 11.2% and 6.7% less (males) than the control group, respectively. Mean body weights and body weight gains of the F1 pups selected for direct test article exposure were further reduced. Mean body weights in the 50 and 150 ppm groups were 18.3% and 17.6% (males) and 18.8% and 21.4% (females) lower than the control group on PND 35, respectively. There were no effects of maternal exposure (pre-weaning) or direct exposure (post-weaning) on mean body weights or body weight gains in the 5 ppm group F1 males and females.
GROSS PATHOLOGY (OFFSPRING)
There were no macroscopic findings in F1 pups that were found dead, euthanized due to the death of the dam or at the scheduled necropsies that could be attributed to maternal test article exposure (pre-weaning) or direct exposure to the test article (post-weaning) at any exposure level.

Any other information on results incl. tables

Table 1: Group mean body weights (g) Males

Study time point		0 ppm	5 ppm	50 ppm	150 ppm
0	Mean SD N	368 13.6 12	368 12.4 12	367 12.1 12	365 14.4 12
1	Mean SD N	389 15.8 12	386 16.2 12	371 17.6 12	357** 25.2 12
2	Mean SD N	403 18.9 12	400 18.7 12	381 21.7 12	363** 26.9 12
3	Mean SD N	419 17.8 12	419 19.7 12	400 25.9 12	376** 29.6 12
4	Mean SD N	446 23.7 12	446 23.0 12	424 27.7 12	390** 34.3 12

** significantly different from control at 0.01 using Dunnett's test

Table2: Group mean body weight changes (g) Males

Study time point		0 ppm	5 ppm	50 ppm	150 ppm
0-1	Mean SD N	22 6.1 12	18 7.4 12	4** 12.9 12	-8** 13.6 12
1-2	Mean SD N	13 7.9 12	14 5.7 12	10 7.9 12	7* 5.6 12
2-3	Mean SD N	17 6.4 12	19 7.8 12	19 6.5 12	13 5.8 12
3-4	Mean SD N	27 6.5 12	27 7.4 12	23 3.9 12	14** 6.5 12

* significantly different from control at 0.05 using Dunnett's test
** significantly different from control at 0.01 using Dunnett's test

Table 3: Group mean body weights (g) Females

Study time point		0 ppm	5 ppm	50 ppm	150 ppm
Week 0	Mean SD N	241 7.8 12	243 8.7 12	242 13.0 12	239 11.4 12
Week 1	Mean SD N	255 7.9 12	250 8.9 12	245 11.7 12	247 10.5 12
Week 2	Mean SD N	262 12.5 12	257 13.0 12	249 14.5 12	252 9.6 12
GD 0	Mean SD N	264 12.1 11	257 14.1 10	256 15.9 11	251 11.1 11
GD 4	Mean SD N	286 11.6 11	282 13.7 10	277 17.6 12	270 10.1 11

GD 7	Mean SD N	297 12.3 11	291 14.2 10	286 18.2 12	275** 10.1 11
GD 11	Mean SD N	318 11.6 11	309 17.4 10	305 20.9 12	293** 11.6 11
GD 14	Mean SD N	333 12.5 11	324 20.8 10	319 20.3 12	307** 12.0 11
GD 17	Mean SD N	362 13.6 11	347 33.5 10	348 20.4 12	327** 13.7 11
GD 20	Mean SD N	408 15.0 11	387 49.4 10	393 25.3 12	357** 19.6 11

** significantly different from control at 0.01 using Dunnett's test
GD – gestation day

Table 4: Group mean body weight changes (g) Females

Study time point		0 ppm	5 ppm	50 ppm	150 ppm
Week 0-1	Mean SD N	14 7.9 12	7 8.8 12	3* 10.1 12	8 10.0 12
Week 1-2	Mean SD N	7 10.1 12	7 6.9 12	4 5.9 12	5 5.1 12
Week 0-2	Mean SD N	21 10.4 12	14 9.0 12	7** 9.6 12	13 11.1 12
GD 0-4	Mean SD N	22 7.1 11	25 6.7 10	22 8.1 11	19 5.7 11
GD 4-7	Mean SD N	12 2.8 11	10 4.2 10	9 3.8 12	5 12.5 11
GD 7-11	Mean SD N	21 4.1 11	18 5.3 10	19 5.1 12	19 14.3 11
GD 11-14	Mean SD N	15 4.7 11	15 4.9 10	14 4.8 12	14 2.1 11
GD 14-17	Mean SD N	29 5.5 11	23 15.0 10	28 3.9 12	20* 5.3 11
GD 17-20	Mean SD N	46 4.4 11	41 17.1 10	45 7.2 12	30* 7.3 11

* significantly different from control at 0.05 using Dunnett's test

Table 5: Group mean body weights (g) Females

Study time point		0 ppm	5 ppm	50 ppm	150 ppm
LD 1-A	Mean SD N	310 17.4 11	298 16.5 9	291* 18.3 12	284** 12.0 11
LD 4-A	Mean SD	321 18.3	313 19.7	308 23.7	301 13.3

	N	11	8	12	11
LD 7	Mean SD N	339 13.4 11	326 18.4 8	318* 21.0 12	305** 19.9 11
LD 14	Mean SD N	358 17.0 11	346 18.6 8	345 17.3 12	327** 18.4 10
LD 21	Mean SD N	359 9.8 11	356 20.3 8	357 20.9 12	334** 18.2 10
LD 28	Mean SD N	313 11.8 11	309 18.0 8	316 14.3 12	311 14.9 10

* significantly different from control at 0.05 using Dunnett's test
** significantly different from control at 0.01 using Dunnett's test
LD – lactation day (A - non-exposed)

Table 6: Group mean body weight changes (g) Females

Study time point		0 ppm	5 ppm	50 ppm	150 ppm
LD 1-4A	Mean SD N	11 12.0 22	17 13.2 8	17 9.9 12	18 8.5 11
LD 4-7	Mean SD N	29 14.2 11	13 7.4 8	10 8.4 12	4 16.4 11
LD 7-14	Mean SD N	19 11.4 11	20 10.8 8	27 9.4 12	18 7.6 10
LD 14-21	Mean SD N	1 11.9 11	10 15.5 8	13 10.2 12	7 9.4 10
LD 21-28	Mean SD N	-46 9.0 11	-47 14.1 8	-41 14.8 12	-23** 11.6 10
LD 1-28	Mean SD N	3 14.0 11	14 10.4 8	25** 13.2 12	28** 14.8 10

Table 7: Summary of food consumption (g/rat/day)

Time point		0 ppm	5 ppm	50 ppm	150 ppm
MALES					
Week 0-1	Mean SD n	26 1.5 12	25 2.0 12	23** 2.1 12	20** 2.6 12
Week 1-2	Mean SD N	25 1.5 12	24 2.2 12	23 2.4 12	21** 2.2 12
FEMALES					
Week 0-1	Mean SD n	18 1.2 12	18 1.1 12	16* 2.0 12	16** 1.7 12
Week 1-2	Mean SD N	19 1.4 12	18 1.3 12	17 1.8 12	17* 1.6 12

* significantly different from the control group at 0.05 using Dunnett's test
** significantly different from the control group at 0.01 using Dunnett's test

Food consumption was not recorded during the breeding period (weeks 2 to 4)

Table 8: Summary of food consumption in Females (g/rat/day)

Time point		0 ppm	5 ppm	50 ppm	150 ppm
GD 0-4	Mean	20	20	19	17*
	SD	2.3	2.1	2.2	2.1
	n	11	10	12	11
GD 4-7	Mean	22	21	22	19**
	SD	2.2	3.3	2.1	1.7
	N	11	10	12	11
GD 7-11	Mean	22	20	21	19**
	SD	2.0	1.0	2.6	1.8
	n	11	10	12	11
GD 11-14	Mean	23	21	23	20
	SD	2.3	2.2	2.6	2.3
	N	11	10	12	11
GD 14-17	Mean	25	22	24	21*
	SD	3.0	4.7	2.5	2.1
	N	11	10	12	11
GD 17-20	Mean	26	23	25	22**
	SD	2.1	4.6	2.2	2.1
	N	11	10	12	11
GD 0-20	Mean	23	21	22	19**
	SD	1.9	2.7	1.8	1.8
	N	11	10	12	11

* significantly different from the control group at 0.05 using Dunnett's test
** significantly different from the control group at 0.01 using Dunnett's test
GD gestation day

Table 9: Summary of food consumption in Females (g/rat/day)

Time point		0 ppm	5 ppm	50 ppm	150 ppm
LD 1-4A	Mean	35	38	38	36
	SD	7.4	8.3	6.7	6.7
	n	11	8	12	9
LD 4-7	Mean	45	44	43	36**
	SD	4.6	7.2	4.9	8.0
	N	11	8	12	11
LD 7-14	Mean	54	53	51	45*
	SD	4.0	10.8	7.6	7.0
	n	11	8	12	10
LD 14-21	Mean	65	62	64	57
	SD	4.5	13.9	7.5	9.4
	N	11	8	12	10
LD 21-28	Mean	113	101	106	97
	SD	13.2	29.2	16.1	22.4
	N	11	8	12	10
LD 1-28	Mean	69	65	66	62
	SD	4.0	15.1	8.3	10.1
	N	11	8	12	8

* significantly different from the control group at 0.05 using Dunnett's test
** significantly different from the control group at 0.01 using Dunnett's tes
LD lactation day
A non exposure period

<u>Overall remarks, attachments</u>

Remarks on results including tables and figures

Table10: Summary of Implantation Sites

Parameter		0 ppm	5 ppm	50 ppm	150 ppm
Implantation sites	Mean SD N	15.3 1.42 11	16.4 1.24 9	16.1 1.83 12	13.9 3.91 11
Number born	Mean SD N	14.5 2.16 11	15.6 1.24 9	15.3 1.92 12	12.9 3.75 11
Unaccounted sites	Mean SD N	0.8 1.78 11	0.9 0.78 9	0.8 0.87 12	1.0 1.61 11

Table 11: Summary of male kidney weight

Parameter		0 ppm	5 ppm	50 ppm	150 ppm
Kidney (g)	Mean SD N	2.87 0.207 12	2.74 0.147 12	2.79 0.220 12	2.59* 0.313 12
Kidney (g/ 100g body weight)	Mean SD N	0.642 0.0549 12	0.607 0.0396 12	0.653 0.0284 12	0.660 0.0575 12
Final body weight	Mean SD N	449 23.9 12	452 24.0 12	427 30.7 12	393** 35.1 12

* Significantly different from the control group at 0.05 using Dunnett's test
** Significantly different from the control group at 0.01 using Dunnett's test

Table 12: Summary of postnatal survival

Time period		0 ppm	5 ppm	50 ppm	150 ppm
PND 0 (relative to number born)	Mean SD N	95.7 8.21 11	97.9 4.47 9	99.5 1.8 12	99.1 3.02 11
PND 0 to PND 1	Mean SD N	100 0.0 11	78.7 40.76 9	99.5 1.7 12	100 0.0 11
PND 1 to PND 4 (pre-selection)	Mean SD N	100 0.0 11	99.1 2.53 8	98.3 4.35 12	99.5 1.68 11
PND 4 (post-selection) to PND 7	Mean SD N	99.1 3.02 11	100 0.0 8	100 0.0 12	97.3 9.05 11
PND 7 to PND 14	Mean SD N	100 0.0 11	100 0.0 8	98.3 5.77 12	97.1 9.04 10
PND 14 to PND 21	Mean SD N	100 0.0 11	100 0.0 8	100 0.0 12	100 0.0 10
PND 21 to PND 28	Mean SD N	100 0.0 11	100 0.0 8	100 0.0 12	100 0.0 10
Birth to PDN 4 (pre-selection)	Mean SD N	95.7 8.21 11	77.1 39.96 9	97.3 5.18 12	98.6 3.3 11
PND 4 (post-selection) to PND 28	Mean SD N	99.1 3.02 11	100 0.0 8	98.3 5.77 12	95.0 15.81 10

PND post natal day

Table 13: Summary of offspring weight (litter as experimental unit)

Study period		0 ppm	5 ppm	50 ppm	150 ppm
PND 1	MALES				
	Mean	7.5	6.9	7.0	7.1
	SD	0.7	0.41	0.57	0.45
	N	11	8	12	11
	FEMALES				
	Mean	7.1	6.8	6.7	6.6
	SD	0.72	0.41	0.45	0.38
	N	11	8	12	10
PND 4 (before selection)	MALES				
	Mean	10.3	9.7	9.2	10.2
	SD	1.51	0.72	1.02	1.80
	N	11	8	12	11
	FEMALES				
	Mean	9.7	9.6	8.7	9.3
	SD	1.5	0.88	0.82	1.39
	N	11	8	12	10
PND 7	MALES				
	Mean	13.5	12.5	12.3	13.3
	SD	2.04	1.53	2.08	2.55
	N	11	8	12	11
	FEMALES				
	Mean	13.1	12.8	11.7	11.9
	SD	1.96	1.55	1.81	1.90
	N	11	8	12	10
PND 14	MALES				
	Mean	23.9	23.1	21.4	22.3
	SD	3.03	2.82	4.37	4.30
	N	11	8	12	10
	FEMALES				
	Mean	23.5	23.4	19.9*	19.5*
	SD	2.97	2.45	3.30	3.47
	N	11	8	12	9
PND 21	MALES				
	Mean	38.0	36.6	34.2	36.4
	SD	5.98	3.64	5.94	7.31
	N	11	8	12	10
	FEMALES				
	Mean	37.6	37.0	31.9*	31.8*
	SD	5.70	3.64	4.17	4.41
	N	11	8	12	9
PND 28	MALES				
	Mean	76.8	72.7	68.3	72.4
	SD	10.20	8.23	11.79	12.66
	N	11	8	12	10
	FEMALES				
	Mean	71.9	69.8	62.8*	62.4*
	SD	8.65	5.75	8.19	8.69
	N	11	8	12	9

* Significantly different from the control group at 0.05 using Dunnett's test
PND post natal day

Table14: Summary of offspring weight (F_1)

Study period		0 ppm	5 ppm	50 ppm	150 ppm
PND 28	MALES				
	Mean	77	75	69	72
	SD	13.6	9.6	12.0	12.8
	N	11	8	12	10
	FEMALES				

	Mean SD N	71 9.6 11	69 5.1 8	63 9.7 12	64 9.3 9
PND 35	MALES Mean SD N FEMALES Mean SD N	 131 22.1 11 117 11.6 11	 123 17.8 8 112 6.7 8	 107* 15.7 12 95** 12.7 12	 108* 18.9 10 92** 12.7 9

* Significantly different from the control group at 0.05 using Dunnett's test
** Significantly different from the control group at 0.01 using Dunnett's test
PND post natal day

Applicant's summary and conclusion

Conclusions

There were no functional effects on reproduction (mating and fertility indices, number of days between pairing and coitus, and gestation length) at any exposure concentration. Therefore, an exposure level of 150 ppm was considered to be the NOAEL (no-observed-adverse-effect level) for parental reproductive toxicity of DMDS when administered via whole-body inhalation exposure to rats. In general, evidence of general toxicity was more pronounced in the F0 males than in the F0 females and consisted of decrements in body weight gain and food consumption in the 50 (males only) and 150 ppm groups. Therefore, the NOAEL for parental systemic toxicity was considered to be 5 ppm. Neonatal toxicity was expressed at 50 and 150 ppm by reduced F1 pup body weights and body weight gains. Mean F1 male and female body weights and body weight gains were reduced further after 1 week of direct DMDS exposure during the postweaning period. Therefore, the NOAEL for neonatal toxicity was 5 ppm.

Executive summary

This study was designed to provide preliminary information on the potential adverse effects of DMDS on male and female reproduction within the scope of a screening study. This encompassed gonadal function, mating behaviour, conception, parturition and lactation of the F_0generation and the development of offspring from conception through day 28 of postnatal life.
Four groups of male and female Crl:CD(SD) rats (12/sex/group) were exposed to either clean filtered air or vapour atmospheres of DMDS, for 6 hours daily for 14 consecutive days prior to mating. DMDS concentrations were 0, 5, 50 and 150 ppm.
Exposure of the F_0males continued during the mating period and through the day prior to euthanasia for a total of 29 days of exposure. The F_0females continued to be exposed throughout mating and gestation through gestation day 20. After parturition, exposure of the F_0females was re-initiated on lactation day 5 and continued through the day prior to euthanasia (lactation day 28). The F_0females that delivered were exposed for a total of 58-72 days. Females with evidence of mating that failed to deliver were exposed through post-mating day 20 for a total of 35-49 days, and then euthanized on post-mating day 25.
The F_1offspring (1/sex/litter) selected for control or DMDS exposure were exposed following weaning, beginning on postnatal day (PND) 28 through PND 34. DMDS concentrations were 0, 5, 50 and 150 ppm.
All animals were observed twice daily for appearance and behaviour. Clinical observations, body weights and food consumption were recorded weekly for F_0males throughout the study and for F_0females prior to mating. During gestation and lactation these parameters were recorded at appropriate intervals. All F_0females were allowed to deliver and rear their pups until lactation day 28. Litters were culled to 10 pups/litter (5/sex, when possible) on PND 4 to reduce the variability among the litters. F_1clinical observations and body weights were recorded on PND 1, 4, 7, 14, 21 and 28 for all pups and also on PND 35 for pups selected for exposure. F_1pups not selected for exposure were necropsied on PND 28, and exposed F_1weanlings were euthanized and discarded without examination on PND 35. F_0males were euthanized following completion of the mating period and F_0females were euthanized on lactation day 28. Complete necropsies were conducted on all F_0animals, and selected organs were weighed. Selected tissues were examined microscopically from all F_0animals in the control and high-exposure groups.

There was one DMDS related early death in the 150 ppm F_0female group on lactation day 7. This female was euthanized following a body weight loss of 39 g and several clinical findings prior death, including a pale body and eyes, shallow respiration and red material around the nose and mouth. A cause of death could not be determined at necropsy or microscopically (with a limited number of tissues being examined).

One F_0female in the 5 ppm group had total litter loss on lactation day 1. No total litter loss occurred at 50 or 150 ppm; therefore, the occurrence at 5 ppm was not considered DMDS related. No DMDS related clinical findings were observed in the F_0males and surviving F_0females. Reproductive performance (mating, fertility, copulation and conception indices), mean numbers of days between pairing and coitus and mean gestation lengths was unaffected by DMDS exposure. There were no signs of dystocia in this study.
Effects on mean body weights, body weight changes and food consumption were noted in the 50 and 150 ppm group F_0males throughout the study and in the 150 ppm group F_0females during gestation and lactation. During the first week of the premating period, a mean body weight loss was noted at 150 ppm, while a reduced mean body weight gain was noted at 50 ppm compared to the control group. Mean body weight gains in the 150 ppm group were reduced for study weeks 2-3 and 3-4 while mean body weight gains in the 50 ppm group were reduced during study week 3-4. Mean F_0maternal body weight gains were reduced during most of the gestation period, and during lactation days 4-7 (following resumption of DMDS exposure).
Mean F_0maternal food consumption was generally reduced in the 150 ppm group females throughout gestation and lactation. There were no effects on body weights, body weight gains or food consumption in F_0males at 5 ppm and in F_0females at 5 and 50 ppm. Mean body weights in the 50 ppm F_0females were lower on lactation days 1 and 7 which was attributed to the reduced mean body weight gains noted during the premating period and not considered evidence of toxicity during lactation. There were no DMDS related macroscopic or microscopic findings in F_0males and females. No DMDS related effect on the number of implantation sites was identified and there were no DMDS effects on organ weights noted in the F_0males or females.
The mean number of pups born and live litter size on PND 0 were slightly lower in the 150 ppm group as a result of a single female, which also had an atypically low number of implantation sites; no relationship to DMDS was evident. No DMDS related effects on the mean number of pups born, live litter size and the percentage of males at birth were observed in the 5 and 50 ppm groups. Postnatal survival in the 150 ppm group was lower during PND 4-28 due to a single female that lost 5 pups during PND 5-9. There were no effects of maternal exposure to DMDS on postnatal survival in the 5 and 50 ppm groups. The general physical condition of the F_1pups was not affected by maternal exposure to DMDS.
There were no clinical findings in the F_1pups selected for 1 week of direct DMDS exposure during the post-weaning period. Mean F_1male and female pup body weight gains were lower in the 50 and 150 ppm groups during PND 4-28 (females) and PND 7-14 (males). Mean body weights and body weight gains of the F_1pups selected for direct DMDS exposure were further reduced. There were no effects of maternal exposure (pre-weaning) or direct exposure (post-weaning) on mean body weights or body weight gains in the 5 ppm group F_1males and females. There were no macroscopic findings in F_1pups that were found dead, euthanized due to the death of the dam or at the scheduled necropsies that could be attributed to maternal DMDS exposure (pre-weaning) or direct exposure to DMDS (post-weaning) at any exposure level.
There were no functional effects on reproduction (mating and fertility indices, number of days between pairing and coitus, and gestation length) at any exposure concentration. Therefore, an exposure level of 150 ppm was considered to be the NOAEL (no-observed-adverse-effect level) for parental reproductive toxicity of DMDS when administered via whole-body inhalation exposure to rats. In general, evidence of general toxicity was more pronounced in the F_0males than in the F_0females and consisted of decrements in body weight gain and food consumption in the 50 (males only) and 150 ppm groups. Therefore, the NOAEL for parental systemic toxicity was considered to be 5 ppm. Neonatal toxicity was expressed at 50 and 150 ppm by reduced F_1pup body weights and body weight gains. Mean F_1male and female body weights and body weight gains were reduced further after 1 week of direct DMDS exposure during the postweaning period. Therefore, the NOAEL for neonatal toxicity was 5 ppm.

3.11.2 **Development toxicity – Teratogenicity**

[Study 1] Nemec (WIL) 2005/K1 KS/Developmental toxicity-teratogenicity, rabbit

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	Key Stduy	1 (reliable without restriction)	Nemec MD	2005	An inhalation prenatal developmental toxicity study of dimethyl disulfide in rabbits

Materials and methods

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 414 (Prenatal Developmental Toxicity Study)	no
according to	EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study)	no

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Purity:
99.8%

Test animals

Species : rabbit

Strain : New Zealand White

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Covance Research Products, Inc., Denver, Pennsylvania
- Age at reception: 5.5 months old
- Gestation age at reception: 1, 2 or 3 days
- Weight at study initiation: 3063 g to 4625 g for the animals in replicate one and 3049 g to 4150 g for animals in replicate two on gestation day 0
- Housing: individually in clean, stainless steel wire-mesh cages suspended above ground corncob bedding
- Diet: PMI Nutrition International, LLC, Certified Rabbit LabDiet® 5322, ad libitum
- Water: Reverse osmosis-purified (on-site) drinking water, ad libitum- Acclimation period: 3-5 days

ENVIRONMENTAL CONDITIONS
- Temperature: 18.7°C to 23.5°C
- Humidity: 49.2% to 63.8%
- Air changes (per hr): 10
- Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration : inhalation: vapour

Type of inhalation exposure (if applicable) : whole body

Vehicle : unchanged (no vehicle)

Details on exposure

The exposures were conducted (in two sequential replicates) in four 2.0 m3 stainless steel and glass whole-body exposure chambers. One chamber was dedicated for each group for the duration of the study. Food and water were withheld during each daily exposure period.Vapors of the test article were generated using glass gas washing bottles contained individually in a Plexiglas cube under slight negative pressure. Nitrogen from a regulated in-house source and monitored by Omega and Cole-Parmer rotameters, flowed through the fritted disc of the gas washing bottle. Concentrated vapors of the test article were then carried to the chamber inlet and mixed with the chamber ventilation air. The control group was exposed to clean, filtered air.

Analytical verification of doses or concentrations : yes

Details on analytical verification of doses or concentrations

Actual exposure concentrations of the test article atmospheres were determined by gas chromatography (GC). Samples of the exposure atmospheres were automatically collected at approximately 35-minute intervals using a sample loop and a computer-controlled multiposition valve.The overall mean concentrations for the first replicate were 15.3 ppm for the 15 ppm group, 45.0 ppm for the 45 ppm group, and 135.0 ppm for the 135 ppm group. The overall mean concentrations for the second replicate were 15.0 ppm for the 15 ppm group, 45.0 ppm for the 45 ppm group, and 134.6 ppm for the 135 ppm group.

Duration of treatment / exposure : Gestation days 6-28 (23 exposures)

Frequency of treatment : 6 hours per day, 7 days per week

Duration of test : until sacrifice on GD 29

Doses / concentrations : 15, 45, 135 ppm

Basis analytical conc.

No. of animals per sex per dose : 24

Control animals : yes, sham-exposed

Further details on study design

- Dose selection rationale: Exposure levels were selected based on results of a range-finding prenatal developmental toxicity study of dimethyl disulfide in rabbits (WIL-160117, 2005). In the main range-finding phase of that study (Phase II) no maternal or developmental toxicity was observed at exposure levels of 1, 5, 25 and 50 ppm. Due to the lack of maternal and developmental toxicity in that phase, an additional 5–day toxicity study was conducted (Phase III) in nonpregnant females at the exposure levels of 75 and 150 ppm of DMDS. Based on the clinical signs (including head bobbing, head held high and eyes partially closed), body weight losses generally throughout the study and sustained reductions in ood consumption in the 150 ppm group for 5 consecutive days, the high exposure level of 135 ppm in the current study was selected because it was expected to induce maternal toxicity.

<u>**Examinations**</u>

Maternal examinations

CLINICAL OBSERVATIONS AND SURVIVAL

All rabbits were observed twice daily, once in the morning and once in the afternoon (at least 7 hours apart) for moribundity and mortality. Individual detailed clinical observations were recorded from gestation days 0 through 29 (prior to exposure during the treatment period). Animals were also observed for signs of toxicity at the midpoint of exposure and approximately 1 hour following exposure. Additional observations were recorded at the beginning (approximately 20-30 minutes) of exposure when necessary (3 days).

BODY WEIGHTS

Individual maternal body weights were recorded on gestation days 0 (by the supplier under non-GLP conditions), 4 and 6-29 (daily). Group mean body weights were calculated for each of these days. Mean body weight changes were calculated for each corresponding interval and also for gestation days 6-10, 10-14, 14-21, 21-29, 6-21, 6-29 and 0-29.

GRAVID UTERINE WEIGHTS

Gravid uterine weight was collected and net body weight (the day 29 body weight exclusive of the weight of the uterus and contents) and net body weight change (the day 0-29 body weight change exclusive of the weight of the uterus and contents) were calculated and presented for each gravid female at the scheduled laparohysterectomy.

FOOD CONSUMPTION

Individual food consumption was recorded on gestation days 6-29 (daily). Food intake was reported as g/animal/day and g/kg/day for the corresponding body weight change intervals.

Ovaries and uterine content

FEMALE THAT ABORTED

The female that aborted in this study was euthanized by an intravenous injection of sodium pentorbital via a marginal ear vein that day and necropsied. The number and location of implantation sites, corpora lutea and the number of viable fetuses were recorded. Gross lesions were saved for possible future histopathologic examination.

GESTATION DAY 29 LAPAROHYSTERECTOMY

All surviving rabbits were euthanized on gestation day 29 by an intravenous injection of sodium pentobarbital via the marginal ear vein. The thoracic, abdominal and pelvic cavities were opened by a ventral mid-line incision, and the contents were examined.The uterus and ovaries were then exposed and excised. The number of corpora lutea on each

ovary was recorded. The trimmed uterus was weighed and opened, and the number and location of all fetuses, early and late resorptions and the total number of implantation sites were recorded. Uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss.

Fetal examinations

FETAL MORPHOLOGICAL EXAMINATION

Each viable fetus was examined externally and individually weighed. The detailed external examination of each fetus included, but was not limited to,an examination of the eyes and palate and external orifices.Crown-rump measurements and degrees of autolysis were recorded for late resorptions.Each viable fetus was subjected to a visceral examination using a modification of the fresh dissection technique to include the heart and major blood vessels. The sex of each fetus was determined by internal examination. Fetal kidneys were examined and graded for renal papillae development. Heads from all fetuses were examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol.Following fixation in alcohol, each fetus was macerated in potassium hydroxide and stained with Alizarin Red S (Dawson, 1926). External, visceral and skeletal findings were recorded as developmental variations or malformations.

Statistics

Mean maternal body weights (absolute and net), body weight changes (absolute and net) and food consumption, gravid uterine weights, numbers of corpora lutea, implantation sites and viable fetuses, and fetal body weights (separately by sex and combined) were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group. Mean litter proportions (percent per litter) of prenatal data (viable and nonviable fetuses, early and late resorptions, total resorptions, pre- and postimplantation loss and fetal sex distribution), total fetal malformations and developmental variations (external, visceral, skeletal and combined) and each particular external, visceral and skeletal malformation or variation weresubjected to the Kruskal-Wallis nonparametric ANOVA test to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$)intergroup variance, the Mann-Whitney U-test was used to compare the test article-treated groups to the control group.

Historical control data

Available.

<u>Results and discussions</u>

Effect levels

Endpoint	**Effect type**	**Effect level**	**Based on**	**Basis for effect level / Remarks**
NOAEC	maternal toxicity	>= 135 ppm (analytical)		Lack of adverse effects on maternal body weight gain, food consumption and survival
NOAEC	developmental toxicity	>= 135 ppm (analytical)		No developmental toxicity was observed up to 135 ppm
NOAEC	teratogenicity	>= 135 ppm (analytical)		No teratogenic effect was observed up to 135 ppm

Maternal toxic effects

no effects

Details on maternal toxic effects

MATERNAL CLINICAL OBSERVATIONS AND SURVIVAL

One female the 45 ppm group aborted on gestation day 26.Clinical observations noted at the beginning (approximately 20-30 minutes) of the 6-hour exposure period included head held high in two and four animals in the 45 and 135 ppm groups, respectively, and wet clear material around the mouth in three animals in the 135 ppm group on the first day of exposure only. However, these findings were not sustained with continuation of exposure and therefore were not considered adverse effects of the test article.

MATERNAL BODY WEIGHTS AND GRAVID UTERINE WEIGHTS

Mean body weights, body weight gains, net body weights, net body weight gains and gravid uterine weights in the 15, 45 and 135 ppm groups were unaffected by test article exposure throughout gestation (days 6-10, 10-14, 14-21, 21-29 and 6-29).
MATERNAL FOOD CONSUMPTION
A statistically significant ($p<0.05$) decrease in food consumption (evaluated as g/animal/day and g/kg/day) was noted in the 135 ppm group on the first day of exposure (gestation day 6-7) when compared to the control group. The reduction in food consumption was considered test article-related, as food consumption in the 135 ppm group remained slightly lower (not statistically significant) than the control group throughout the first 2 weeks of exposure (gestation days 6-10, 10-14, 14-21 and 6-21), but was not considered adverse because of the lack of concomitant effects on mean bodyweights and body weight gains. During gestation days 21-29 food consumption in the 135 ppm group was similar to the control group. Food consumption in the 15 and 45 ppm groups was unaffected by exposure.
MATERNAL NECROPSY DATA
One, three, six (including the female that aborted) and six females in the control, 15, 45 and 135 ppm groups, respectively, had dark red discoloration of or dark red areas on the lungs (generally all lobes).

Embryotoxic / teratogenic effects

no effects

Details on embryotoxic / teratogenic effects

GESTATION DAY 29 LAPAROHYSTERECTOMY
Intrauterine growth and survival were unaffected by test article administration at exposure levels of 15, 45 and 135 ppm. Parameters evaluated included postimplantation loss, live litter size, fetal body weights and fetal sex ratios. Mean numbers of corpora lutea and implantation sites and the mean litter proportions of preimplantation loss were similar across all groups.
EXTERNAL MALFORMATIONS AND VARIATIONS
There were no external malformations or developmental variations noted in the 15, 45 and 135 ppm groups. In the control group, two fetuses had umbilical herniation of the intestine (several loops of the intestine protruded through an opening in the umbilicus).
VISCERAL MALFORMATIONS AND VARIATIONS
Soft tissue malformations were observed in 3(3), 2(1), 3(1) and 3(2) fetuses (litters) in the control, 15, 45 and 135 ppm groups, respectively. One, one, three and two fetuses in the same respective groups had lobular agenesis of the lungs (absent right accessory lobe).One fetus each in the control, 15 and 135 ppm groups had hydrocephaly (increased cavitation of both the lateral and third ventricles). One fetus in the control group had lobular dysgenesis in the lungs (all lobes small; right lobes fused). These findings occurred similarly in the control group and the proportional values were within the range of WIL historical control data, and were not considered exposure-related.Soft tissue developmental variations occurred in all groups, including the control group, and consisted primarily of blood vessel variations (the left carotid artery arose from the brachiocephalic trunk, right subclavian artery coursed retroesophageal rejoined aortic arch adjacent to ductus arteriosis with no brachiocephalic trunk, right carotid and subclavian arteries arose independently from the aortic arch with no brachiocephalic trunk), accessory spleens, retrocaval ureter, and small or absent gallbladder. Other soft tissue developmental variations observed in the test article-exposed groups occurred infrequently, occurred similarly in the control group or the values were within the range of WIL historical control data, therefore no test article-related effects were evident.
SKELETAL MALFORMATIONS AND VARIATIONS
Skeletal malformations were observed in 6(3), 3(3) and 2(2) fetuses (litters) in the control, 15 and 45 ppm groups, respectively. Two, two and one fetuses in the control, 15 and 45 ppm groups, respectively, had vertebral anomalies with or without associated rib anomalies consisting of absent and extra arches, centra and/or ribs, mislocated centra and fused ribs and centra. Rib anomalies consisting of extra, fused or forked ribs were noted for three and one fetuses in the control and 45 ppm groups, respectively. One fetus each in the 15 and 45 ppm groups had skull anomalies consisting of medially fused nasal or frontal bones. One control group fetus had a costal cartilage anomaly (bifurcated right costal cartilage with the posterior fork associating with the sternum, causing subsequent costal cartilages to associate with the sternum higher than normal). None of the proportional values were statistically significant compared to the control group and the values were within the WIL historical control data ranges. No skeletal malformations were noted at the 135 ppm exposure level and none occurred at a higher incidence than in the control group; therefore, the skeletal malformations were not considered exposure-related. The percent per litter of 13th full ribs in the 45 and 135 ppm groups (44.8% and 55.5% per litter, respectively) were increased compared to the control group (31.3% per litter). Although the difference was statistically significant ($p<0.05$) for the 135 ppm group, the values were within the WIL historical control data range (19.4% - 59.1% per litter) and in the absence of other indicators of developmental toxicity was not considered related toexposure. Additionally, the percent per litter value of 7th cervical ribs in the 45 ppm group (7.9% per litter) exceeded the maximum mean value in the WIL historical control data (7.7% per litter); however, this

increase did not occur in an exposure-related manner (3.1% per litter in the 135 ppm group) and was not considered exposure-related. Other skeletal developmental variations occurred in all groups, including the control group, and consisted of sternebra (e) nos. 5 and/or 6 unossified, bent hyoid arches, 13th rudimentary rib(s), accessory skull bones and 27 presacral vertebrae. The mean litter percent of the skeletal variants observed in the exposure groups occurred similarly to the control group or were within the range of the WIL historical control data. No relationship to test article exposure was evident.

Applicant's summary and conclusion

Conclusions

Transient clinical observations were noted at 45 and 135 ppm on the first day of exposure only and decreased food consumption at 135 ppm was sustained throughout the first 2 weeks of exposure but not considered adverse. Additionally, a macroscopic finding of dark red discoloration of or dark red areas on the lungs (generally all lobes) was noted at all exposure levels. No developmental toxicity was observed in any fetuses in this study.Therefore, an exposure level of 135 ppm was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity (based on the lack of adverse effects on maternal body weight gain, food consumption and survival) and an exposure level of 135 ppm was considered to be the NOAEL for developmental toxicity when dimethyl disulfide was administered via whole-body inhalation to rabbits and developmental toxicity when dimethyl disulfide was administered via whole-body inhalation to rabbits.

Executive summary

Dimethyl disulphide (DMDS) was evaluated in an inhalation prenatal developmental toxicity study in rabbits performed following the OECD guidleine # 414. DMDS was administered via whole-body inhalation as avapor to three groups of 24 time-mated female New Zeeland White rabbits on a 6-hoursper day basis during gestation days 6 through 28. A concurrent control group of 24 time-mated rabbits were exposed to clean, filtered air on a comparable regimen. The DMDSexposure concentrations were 0, 15, 45 and 135 ppm.
Transient clinical observations were noted at 45 and 135 ppm on the first day of exposure only and decreased food consumption at 135 ppm was sustained throughout the first 2 weeks of exposure but in the absence of effects on maternal body weight gains these findings were not considered adverse. Food consumption in the 15 and 45 ppm groups and mean body weights,body weight gains, net body weights, net body weight gains and gravid uterine weights in the 15, 45 and 135 ppm groups were unaffected by DMDS exposure for the duration of the study. A macroscopic finding of dark red discoloration of or dark red areas on the lungs (generally all lobes) was noted at all exposure levels. Intrauterine growth and survival was unaffected by test article exposure and no test article related malformations or developmental variations were noted at any exposure level.
An exposure level of 135 ppm was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity (based on the lack of adverse effects on maternal body weight gain, food consumption and survival) and an exposure level of 135 ppm was considered to be the NOAEL for developmental toxicity when dimethyl disulfide was administered via whole-body inhalation to rabbits.

[Study 2] Nemec (WIL) 2006/K1 KS/ Developmental toxicity-teratogenicity, rat

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key Stduy	1 (reliable without restriction)	Nemec MD	2006	Inhalation prenatal developmental toxicity study of dimethyl disulfide in rats

Materials and methods

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 414 (Prenatal Developmental Toxicity Study)	no

according to	EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study)	no

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.8%

Test animals

Species : rat

Strain : Sprague-Dawley

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Laboratories, Inc., Raleigh, North Carolina,
- Age: 70 days old upon receipt.
- Weight at study initiation: 218 g to 290 g on gestation day 0.
- Housing: individually housed in clean, stainless steel wire-mesh cages suspended above cage-board.
- Diet : PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002, ad libitum
- Water : Reverse osmosis-purified (on-site) drinking water, ad libitum
- Acclimation period: 14 days

ENVIRONMENTAL CONDITIONS (NON-EXPOSURE PERIODS)
- Temperature : 22°C ± 3°C
- Humidity: 50% ± 20%
- Air changes (per hr): 10
- Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration : inhalation: vapour

Type of inhalation exposure (if applicable) : whole body

Vehicle : unchanged (no vehicle)

Details on exposure

TREATMENT REGIMEN
Exposures were conducted in 2.0-m3 stainless steel and glass whole-body exposure chambers. The chambers were operated under dynamic conditions, at a slight negative pressure with approximately 12-15 air changes per hour.
EXPOSURE ATMOSPHERE GENERATION AND MONITORING
Vapors of the test article were generated using a bubbler type (gas washing bottle) vaporization system. The test article vapors were then directed to the exposure chamber inlet where vapor concentration was reduced to the desired level by mixing with the chamber ventilation air. The control group was exposed to clean, filtered air.

Analytical verification of doses or concentrations : yes

Details on analytical verification of doses or concentrations

Actual exposure concentrations within each chamber (approximate animal-breathing zones) were measured at least 10 times (approximately every 35 minutes) during each daily exposure period by a validated gas chromatographic method. At least 1 standard was analyzed each day prior to exposure to confirm gas chromatographic calibration.Actual exposure concentrations within each chamber (approximate animal-breathing zones) were measured at least 10 times (approximately every 35 minutes) during each daily exposure period by a validated gas chromatographic method. At least 1 standard was analyzed each day prior to exposure to confirm gas chromatographic calibration. Overall mean

measured exposure concentrations were 0.0, 5.0, 20.2 and 80.0 ppm for the same respective target exposures.

Details on mating procedure

At the conclusion of the acclimation period, all available females (approximately 12 weeks old) were weighed and examined in detail for physical abnormalities. Each animal judged to be in good health and meeting acceptable body weight requirements (a minimum of 220 g) was placed in a suspended wire-mesh cage with a resident male from the same strain and source for breeding. Resident males were untreated, sexually mature rats utilized exclusively for breeding. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm in a vaginal lavage. The day on which evidence of mating was identified was termed gestation day 0.

Duration of treatment / exposure : gestation days 6-19

Frequency of treatment : 6 hours per day

Duration of test : sacrifice on GD 20

Doses / concentrations : 5, 20 and 80 ppm

Basis analytical conc

No. of animals per sex per dose : 27

Control animals : yes, sham-exposed

Examinations

Maternal examinations

CLINICAL OBSERVATIONS AND SURVIVAL: yes

All rats were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality. Individual detailed clinical observations were recorded from gestation days 0 through 20 (prior to exposure during the treatment period). Animals were also observed for signs of toxicity at the midpoint of exposure (if visible through the chamber windows) and approximately 1 hour following completion of exposure. All significant findings were recorded.BODY WEIGHT: Yes- Time schedule for examinations: gestation days 0 and 6-20 (daily).

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): Yes

- Time schedule : gestation days 0 and 6-20 (daily)
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes

WATER CONSUMPTION : no

POST-MORTEM EXAMINATIONS: Yes

- Sacrifice on gestation day 20
- Thoracic, abdominal and pelvic cavities were opened by a ventral mid-line incision, and the contents were examined.

Ovaries and uterine content

LAPAROHYSTERECTOMY

- The number of corpora lutea on each ovary was recorded.
- The trimmed uterus was weighed and opened, and the number and location of all fetuses, early and late resorptions and the total number of implantation sites were recorded. The individual uterine distribution of implantation sites was documented. The placentae were also examined.

GRAVID UTERINE WEIGHTS

Gravid uterine weight was collected and net body weight (the gestation day 20 body weight exclusive of the weight of the uterus and contents) and net body weight change (the gestation day 0-20 body weight change exclusive of the weight of the uterus and contents) were calculated and presented for each gravid female at the scheduled laparohysterectomy.

Fetal examinations

- External examinations: Yes, all per litter
- Soft tissue examinations: Yes, all per litter
- Skeletal examinations: Yes, all per litter
- Head examinations: Yes, one-half per litter in Bouin's fixative for subsequent soft-tissue examination by the Wilson sectioning technique. The heads from the remaining one-half of the fetuses were examined by a mid-coronal slice.

Statistics

Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test article-exposed group to the control group.Mean maternal body weights (absolute and net), body weight changes (absolute and net) and food consumption, gravid uterine weights, numbers of corpora lutea, implantation sites and viable fetuses, and fetal body weights (separately by sex and combined) were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant (p<0.05) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test article-exposed groups to the control group. Mean litter proportions (percent per litter) of prenatal data (viable and nonviable fetuses, early and late resorptions, total resorptions, pre- and postimplantation loss, and fetal sex distribution), total fetal malformations and developmental variations (external, visceral, skeletal and combined) and each particular external, visceral and skeletal malformation or variation were subjected to the Kruskal-Wallis nonparametric ANOVA test to determine intergroup differences. If the ANOVA revealed statistically significant (p<0.05) intergroup variance, Dunn's test (Dunn, 1964) was used to compare the test article-exposed groups to the control group.

Results and discussions

Effect levels

Endpoint	**Effect type**	**Effect level**	**Based on**	**Basis for effect level / Remarks**
NOAEC	maternal toxicity	20 ppm (analytical)		lower mean maternal body weight gains and food consumption at 80 ppm,
NOAEC	developmental toxicity	20 ppm (analytical)		lower mean fetal weight and increased mean litter proportions of several skeletal variations at 80 ppm,
NOAEC	teratogenicity	>= 80 ppm (analytical)		No malformation observed up to 80 ppm

Maternal toxic effects : yes

Details on maternal toxic effects

MATERNAL CLINICAL OBSERVATIONS AND SURVIVAL

All females in the control, 5, 20 and 80 ppm groups survived to the scheduled necropsy on gestation day 20. No test article-related clinical findings were noted at the daily examinations, at the midpoint of exposure or 1 hour following the exposure period at any dosage level.

MATERNAL BODY WEIGHTS AND GRAVID UTERINE WEIGHTS

A test article-related mean body weight loss during gestation days 6-9 and lower mean body weight gains during gestation days 9-12, 12-20 and 6-20 (the entire exposure period) were noted in the 80 ppm group compared to the control group; the differences were statistically significant (p<0.01). The decrements in body weight gain throughout the exposure period resulted in mean body weights that were 5.6% to 9.5% lower (p<0.01) than control group values during gestation days 10-20. Also in the 80 ppm group, mean net body weight was 8.5% lower and mean net body weight gain was lowercompared to control group values; the differences were statistically significant (p<0.01). Mean gravid uterine weight at 80 ppm was also statistically significantly (p<0.01) lower than the control group value, corresponding to lower mean fetal weights observed in this group.In the 20 ppm group, mean body weight gains were slightly lower than the control group during gestation days 6-9 and 9-12; the differences were statistically significant (p<0.05 or p<0.01). Mean body weight gains in the 20 ppm group were similar to control groupvalues during gestation days 12-20 and when the entire exposure period (gestation days 6-20) was evaluated. The test article-related lower mean body weight gains in this group noted during the first week of exposure were not of sufficient magnitude to result in lower mean body weights. Mean net body weight, net body weight gain and gravid uterine weight in this group were similar to control group values.Mean maternal body weights, body weight gains, net body weight, net body weight gain and gravid uterine weight in the 5 ppm group were unaffected by test article exposure. Differences from the control group were slight and generally not statistically significant.MATERNAL

FOOD CONSUMPTION

Mean food consumption, evaluated as g/animal/day and g/kg/day, in the 80 ppm group was statistically significantly lower (p<0.01) than control group values during gestation days 6-9, 9-12, 12-20 and when the entire exposure period (gestation days 6-20) wasevaluated. These decrements in food consumption were considered test article-related and corresponded to the mean body weight loss and lower body weight gains noted for this group during the same intervals.In the 20 ppm group, mean food consumption was similar to the control group during gestation days 6-9, but test article-related, slightly lower (p<0.05 or p<0.01) mean food consumption was noted during gestation days 9-12. This transient decrease in mean food consumption corresponded to the lower mean body weight gain at 20 ppm from gestation days 9-12. Examination of food consumption and body weight data for individual animals during this interval revealed no consistent trends linking low food consumption to reduced body weight gain (or body weight loss) at 20

ppm. Based on the variability of the individual animal data and the similarity of mean food consumption values to control group values during gestation days 12-20 and over the entire exposure period (gestation days 6-20), the slightly lower mean food consumption noted from gestation days 9-12 was not considered adverse at this exposure level.Food consumption in the 5 ppm group was unaffected by test article exposure. Differences from the control group were slight and generally not statistically significant.
MATERNAL NECROPSY DATA
At the scheduled necropsy on gestation day 20, no test article-related internal findings were observed at exposure levels of 5, 20 and 80 ppm.

Embryotoxic / teratogenic effects : yes

Details on embryotoxic / teratogenic effects

GESTATION DAY 20 LAPAROHYSTERECTOMY DATA
Mean fetal weight in the 80 ppm group (3.0 g) was lower than the concurrent control group value (3.7 g) and the minimum mean value in the WIL historical control data (3.4 g). The difference from the concurrent control group was statistically significant (p<0.01) and was considered test article-related. Postimplantation loss, live litter size and fetal sex ratios in the 80 ppm group were unaffected by maternal test article exposure.Intrauterine growth and survival were unaffected by the test article at exposure levels of 5 and 20 ppm. Mean numbers of corpora lutea and implantation sites and the mean litter proportions of preimplantation loss were similar across all groups.
FETAL MORPHOLOGICAL DATA
The numbers of fetuses (litters) available for morphological evaluation were 405(27), 405(26), 406(27) and 408(26) in the control, 5, 20 and 80 ppm groups, respectively. Malformations were observed in 2(2), 1(1), 2(2) and 2(1) fetuses (litters) in these same respective exposure groups.
EXTERNAL MALFORMATIONS AND VARIATIONS
External malformations were noted for 1, 0, 1 and 2 fetuses in the control, 5, 20 and 80 ppm groups, respectively, and included the following. Microphthalmia (left orbit appeared smaller than normal) in fetus no. 31383-03 in the control group and fetus nos. 31480-13 and 31480-18 in the 80 ppm group. Fetus no. 31480-13 also had anal atresia and vertebral agenesis (all vertebrae posterior to lumbar vertebra no. 4 absent). The only other external malformation observed in this study, localized fetal edema (neck and thorax), was noted for fetus no. 31402-17 in the 20 ppm group. Because theseexternal malformations were observed in single fetuses, were also observed in the control group, and/or were observed in a manner that was not related to maternal exposure concentration, none were considered test article-related.No external developmental variations were noted for fetuses in this study.
VISCERAL MALFORMATIONS AND VARIATIONS
Visceral malformations consisted of hydrocephaly (increased cavitation of both lateral ventricles and the third ventricle) in control group fetus no. 31467-06, and a malpositioned esophagus (located to the right of the trachea) and lobular dysgenesis of the lungs (all right lobes were fused) in fetus no. 31478-03 in the 5 ppm group. Because no soft tissue malformations were noted in the 20 and 80 ppm groups, the soft tissue malformations noted at 5 ppm were not considered test article-related.Visceral developmental variations noted in the 5 and 20 ppm groups consisted of renal papillae not developed (Woo and Hoar grade 0) and/or distended ureters, and an accessory spleen. These variations were not considered test article-related because there were no visceral developmental variations noted for fetuses in the 80 ppm group.Renal papillae not fully developed (Woo and Hoar grade 1) were observed in 1 fetus each in the control, 5 and 20 ppm groups (fetus nos. 31395-05, 31391-06 and 31517-06, respectively). Atrial cysts and a white area on the right atrium were noted for fetus no. 31453-11 in the control group. These findings were not classified as either malformations or developmental variations and were not included in any tabulation.SKELETAL
MALFORMATIONS AND VARIATIONS
The only fetal skeletal malformation in this study, sternoschisis (sternal band nos. 1-6 not joined), was noted for a single fetus (no. 31444-04) in the 20 ppm group. Because no skeletal malformations were noted at 80 ppm, this malformation was not considered test article-related.Test article-related differences in the mean litter proportions of skeletal developmental variations were noted in the 80 ppm group. These differences included increased mean litter proportions of unossified sternebrae nos. 5 and/or 6, unossified sternebrae nos. 1, 2, 3 and/or 4, reduced ossification of the vertebral arches, unossified pubis and unossified hyoid, and a decreased mean litter proportion of ossified cervical centrum no. 1 at 80 ppm. Only the difference for unossified sternebrae nos. 5 and/or 6 was statistically significant (p<0.01) compared to the concurrent control group. These skeletal variations were considered test article-related because they corresponded to the reduced mean fetal body weight at 80 ppm, indicative of developmental delay, and were occasionally outside of the WIL historical control data range. The mean litter proportions of the test article-related skeletal developmental variations are summarized in the text table.The mean litter proportions of malaligned sternebrae in the 5, 20 and 80 ppm groups (1.0%, 1.7% and 1.3% per litter, respectively) were higher than the concurrent control group value (0.3% per litter), but did not exceed the range of mean values in the WIL historical control data (0.0% to 1.7% per litter) and did not occur in a manner that was exposure-related. Therefore, malaligned sternebrae in these groups were not considered test article-related. Skeletal developmental variations noted in the 5 and 20 ppm groups consisted primarily of

unossified sternebrae nos. 5 and/or 6, ossified cervical centrum no. 1, and 14th rudimentary ribs. These variations were not considered test article-related because the mean litter proportions in these groups were similar to control group values.

Any other information on results incl. tables

Text table. mean litter proportions of the test article-related skeletal developmental variations

Finding	0 ppm	5 ppm	20 ppm	80 ppm	WIL HC Mean (Range)
Sternebrae # 5 and/or 6 unossified	22.2	18.3	23.4	51.0++	7.4 (0.3-23.1)
Sternebrae # 1, 2, 3 and/or 4 unossified	0.2	0.2	0.5	4.3	0.2 (0.0-1.3)
Cervical centrum # 1 ossified	26.5	21.6	22.1	15.5	19.4 (6.6-34.4)
Reduced ossification of the vertebral arches	0.2	0.0	0.5	2.2	0.1 (0.0-1.1)
Pubis unossified	0.0	0.0	0.2	0.7	0.1 (0.0-2.3)
Hyoid unossified	0.2	0.2	0.0	0.7	1.5 (0.0-4.2)

HC = Historical control
++ = p0.01

Applicant's summary and conclusion

Conclusions

Based on lower mean maternal body weight gains and food consumption noted at 80 ppm, a dosage level of 20 ppm was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity. Based on the lower mean fetal weight and increased mean litter proportions of several skeletal variations noted at 80 ppm, an exposure level of 20 ppm was also considered to be the NOAEL for prenatal developmental toxicity when pregnant Crl:CD(SD) rats were exposed to dimethyl disulfide via whole-body inhalation exposure for 6 hours daily during gestation days 6-19.

Executive summary

ln a developmental toxicity study performed following the OECD guideline # 414, four groups of 27 bred femaleCrl:CD(SD) rats were exposed to either filtered or vapor atmospheres of DMDS for6 hours daily in whole-body inhalation chambers during gestation days 6 through 19.Test concentrations were 0,5,20 and 80 ppm. All animals were observed twice daily formortality and moribundity. Clinical observations, body weights and food consumption were recorded at appropriate intervals. On gestation day 20, a laparohysterectomy was performed on each female. The uterus, placenta and ovaries were examined and thenumber of fetuses, early and late resorptions, total implantations and corpora lutea wererecorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were culculated. The fetuses weree weighed, sexed and examined for external, visceral and skeletal malformations and developmental variations. Thematernal LOAEL was 80 ppm based on lower mean maternal body weight gains and foodconsumption. The NOAEL for maternal toxicity was 20 ppm. The feta/developmentaltoxicity LOAEL was also 80 ppm based on lower mean fetal weight and increased meanlitter proportions of several skeletal variations. The NOAEL for fetal/developmental toxicity was 20 ppm, no teratogenic effect was observed.

[Study 3] Barker (Hazleton-UK) 1991/K1 SS/Developmental toxicity-teratogenicity, rat

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Supporting Stduy	1 (reliable without restriction)	Barker L	1991	Dimethyl Disulfide (DMDS), Inhalation Teratology|Study in the Rat

Materials and methods

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 414 (Prenatal Developmental Toxicity Study)	

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.88%

Test animals

Species : rat

Strain : Sprague-Dawley

Details on test animals and environmental conditions
TEST ORGANISMS:
- Source: Charles River UK Ltd., Margate
- Age at reception: 10-12 weeks
- Weight at reception: 165-218 g
- Number of animals: 100 rats : 25 females / dose group (3 dose groups + 1 control group)
- Acclimatation period: no data

HOUSING
The animals were housed in group of 6 in suspended stainless steel cages.
FOOD and WATER
- Food: SQC rat and Mouse breeder Diet No. 3 ad libitum excepted during exposure
- Water: filtered tap water, ad libitum excepted during exposure

ENVIRONMENTAL CONDITIONS
- Temperature : 19-25°C
- Relative humidity : 40-70%
- Light/dark cycle : 12h/12h
- Ventilation : 15 air changes/hour

Administration / exposure

Route of administration : inhalation

Type of inhalation exposure (if applicable) : whole body

Vehicle : unchanged (no vehicle)

Details on exposure
- Production of test atmospheres:
Four horizontal flow, recirculating exposure chambers were used. Each was made of stainless steel with perspex (Plexiglas) doors and a fan to mix the atmospheres by recirculation. The compressed air supply was from a clean, dry, filtered source. The 3 concentrations of test article vapour were produced by passing metered flows of air through sintered glass frits immersed in separate containers of test article. The resulting outputs of vapour were introduced to the diluent air inlet duct of each test chamber. Mixing, within the duct and recirculation system, ensured the production of homogeneous atmospheres for animal exposure. The chambers were ventilated at a rate of at least 12 air changes per hour. Air flows were monitored continuously and recorded twice hourly during exposure. The exhaust streams were purified with activated charcoal and vented to the outside of the building. - Vehicle: filtered air

Analytical verification of doses or concentrations : yes

Details on analytical verification of doses or concentrations

- Exposure chamber test article concentration * Measured concentration Samples for analysis were withdrawn from the exposure chambers twice hourly through sample lines leading from each chamber through a sampling valve into a total hydrocarbon analyser. The analysis was performed with an Analysis Automation total hydrocarbon analyser type 523 Detector with a Flame ionisation detector (FID) . The chamber concentrations of the test article were close to target values throughout the exposure period.* Nominal concentrationThe total weight of test article used and total volume of diluent air were measured for each exposure.
Group / Target / Nominal / Analytical concentrations
1 / 0
2 / 5 / 9.27 / 5.17 ppm
3 / 15 / 26.71 / 15.35 ppm
4 / 50 / 86.59 / 50.46 ppm

Duration of treatment / exposure : day 6 to day 15 of gestation

Frequency of treatment : 6 h/day

Duration of test : up to gestation day 20

Doses / concentrations : 5, 15, 50 ppm

Basis analytical conc.

No. of animals per sex per dose : 30

Control animals : yes, sham-exposed

Further details on study design

Three groups of 30 mated female rats were exposed to DMDS by whole body exposure at 5, 15 or 50 ppm for 6 hours daily from day 6 to day 15 of gestation. A similar group of 30 rats, exposed to filtered air only over the same period, served as controls. All animals were maintained until day 20 of gestation, killed and their uterine content assessed.

Examinations

Maternal examinations

- Morbidity and mortality
All females were examined twice daily to detect any which were dead or moribund.
- Clinical observations
All females were examined daily from day 3 to day 20 of gestation. Any abnormalities of appearance or behaviour or other signs of reaction to treatment or ill health were recorded.
- Body weight
The body weight of each female was recorded on days 3, 6, 10, 15, and 20 of gestation.
- Food intake The amount of food consumed by each cage of females was recorded daily from day 3 to day 20 of gestation and reported on the body weight intervals.
- Terminal studies
* Necropsy: All females were killed on day 20 of gestation, in random group order, by cervical dislocation and examined macroscopically.

Ovaries and uterine content

The ovaries and uteri were removed and examined and the following data recorded: pregnancy status number of corpora lutea number and intrauterine position of implantations subdivided into: live foetuses, early intrauterine deaths, late intrauterine deaths and dead foetuses.

Fetal examinations

Live foetuses were killed by an intracardiac injection of sodium pentobarbitone solution (200 mg/ml). Foetuses were weighed individually, examined externally and sexed. Approximately one half of the foetuses in each litter (selected by systematic sampling) were dissected and the viscera were examined. They were then eviscerated and the carcasses processed to stain the ossified skeleton (Alizarin technique). The skeleton was examined and preserved and stored in absolute glycerol (containing thymol crystals). The remaining foetuses were placed in Bouin's fluid for at least two weeks to allow fixation and partial decalcification. They were then transferred to 70% industrial methylated spirit. At examination, the head was removed by a coronal cut through the mouth, pharynx and back of the head and sections of the head were examined. The remaining portion of the foetus was examined by dissection and was preserved, with the

head sections, in Bouin's fluid and stored in plastic vials. Foetal abnormalities were recorded as malformations (rare and/or potentially lethal defects) and variations (cormnonly occurring non-lethal abnormalities).

Statistics

The following parameters were analysed:Body weight, body weight gain, food intake, litter weight : analysis of variance, Dunnett's testFertilitiy indices: Cochran-Armitage test and Fisher-Irwin exact test with Bonferroni adjustmentCorpora lutea, implantations, foetuses, pre-implantation loss and post-implantation loss, malformation, foetal weight, percentage male foetuses: Kruskal-Wallis non-parametric Anonva, Wilcoxon's rank-sum test and Terpstra-Jonkcheere test.malformations

Indices

Percentage pre-implantation loss, percentage post-implantation loss and percentage male foetuses

Historical control data

Available.

Results and discussions

Effect levels

Endpoint	Effect type	Effect level	Based on	Basis for effect level / Remarks
NOAEC	maternal toxicity	5 ppm (analytical)		Dose-related reductions in maternal weight gain at 15 and 50 ppm
NOAEC	teratogenicity	> 50 ppm (analytical)		No malformation observed up to 50 ppm
NOAEC	other: embryotoxicity/foetotoxicity	15 ppm (analytical)		Reduced litter and foetal weights at 50 ppm

Maternal toxic effects : yes

Details on maternal toxic effects

MORBIDITY/MORTALITY
There were no deaths.
CLINICAL CONDITIONS
A higher incidence of rough haircoat was observed at 50 ppm. Clinical condition at 5 and 15 ppm did not differ from controls.
BODY-WEIGHT
Dosage-related reductions in weight gain were observed at 15 and 50 ppm. At 50 ppm, weight gain was 40% lower than control over the exposure period (day 6 to 15, p < 0.001). At 15 ppm, weight gain over the exposure period was 16% lower than control group days 6 to 15, p< 0.01)
FOOD CONSUMPTION
Food intake was lower (p< 0.001) than controls at 50 ppm but comparable at 5 or 15 ppm.
NECROPSY FINDINGS
No unusual lesions were observed at necropsy. Pregnancy rates were lower than expected (73.3, 50.0, 86.7 and 70.0% at 0, 5, 15 and 50 ppm, respectively), but sufficient data was available in all groups for adequate assessment of the study results.
CAESARIAN DATA
- Corpora lutea and implatations: No effects
- Pre implantation loss: Higher than control at 5 and 50 ppm.
- Post implantation loss: No effect.
- Litter size: Smaller than expected in all groups (12.0 to 14.0).

Embryotoxic / teratogenic effects : yes

Details on embryotoxic / teratogenic effects

FOETAL DATA

- Sex ratioNo effect.
- Litter and foetal weights
Reduced at 50 ppm. At 5 and 15 ppm these parameters were comparable to controls.
- Malformations
No malformations were observed in foetuses from the treated groups.
- Variations
A slightly higher incidence of retarded ossification was observed at 50 ppm but was considered to indicate delayed maturation, as a result of the lower foetal weight, rather than a teratogenic effect.

Any other information on results incl. tables

Table 1: Summary of body weight and changes during gestation (g)

Day of gestation	0 ppm	5 ppm	15 ppm	50 ppm
3	209	209	206	208
6	231	232	228	226
10	260	262	251**	241***
15	297	297	283**	265***
20	351	360	346	327
% weight change days 3-20	67.9	72.2	68.0	57.2
% weight change days 6-15	28.6	28.0	24.1**	17.3***

** body weight gain significantly lower than control days 6-10, 6-15 (p<0.01 analysis of variance, Dunnett's test)
*** body weight gain significantly lower than control days 6-10, 6-15, 10-15 (p<0.001 analysis of variance, Dunnett's test)

Table 2: Summary of food intake (g/animal/day)

Days of gestation	0 ppm	5 ppm	15 ppm	50 ppm
3-6	25	25	26	25
6-10	25	24	24	21***
10-15	26	25	25	21***
15-20	28	27	30	27
Mean intake (g/day) days 3-20	26	25	26	23
Mean intake (g/day) days 6-15	26	25	25	21***

*** significantly lower than control (p<0.001, analysis of variance, Dunnett's test)

Table 3 : Summary of uterine/implantation data

Group mean caesarian data	0ppm	5 ppm	15 ppm	50 ppm
Number of females with live foetuses at day 20 gestation	22	15	26	21
Number of corpora lutea	299	215	353	289
Mean number per female	13.6	14.3	13.6	13.8
Number of implantations	264	174	310	244
Mean number per female	12.0	11.6	11.9	11.6
% pre-implantation loss	11.7	19.1	12.2	15.6
Number of early intrauterine deaths	5	12	13	10
Mean number per female	0.2	0.8	0.5	0.5
Number of late intrauterine deaths	1	0	2	0
Mean number per female	0.0	0.0	0.1	0.0
Number of dead foetuses	0	0	0	0
Mean number per female	0.0	0.0	0.0	0.0
%post-implantation loss	2.3	6.9	4.8	4.1
Number of foetuses	258	162	295	234
Mean number per female	11.7	10.8	11.3	11.1

% of implantations	97.7	93.1	95.2	95.9

Table4: Summary of number of foetuses and litter weights (g)

	0 ppm	**5 ppm**	**15 ppm**	**50 ppm**
Number of male foetuses	128	73	152	119
Number of female foetuses	130	89	143	115
%male foetuses	49.6	45.1	51.5	50.9
Mean litter weight	43.7	41.8	41.7	38.8
Mean foetal weight	3.8	3.9	3.7	3.5**
Mean foetal weight males only	4.0	4.1	3.8	3.6**
Mean foetal weight females only	3.7	3.7	3.6	3.4**

** significantly different from control at 0.01 by non-parametric ANOVA and Wilcoxon rank-sum test

Table 5:foetal defect data

	0 ppm	5 ppm	15 ppm	50 ppm
EXTERNAL AND VISCERAL DEFECTS				
Number of foetuses examined	258	162	295	234
Number showing malformations	1	0	0	0
% of foetuses examined	0.4	0.0	0.0	0.0
Number showing variations	52	27	65	52
% of foetuses examined	20.2	16.7	22.0	22.2
SKELETAL DEFECTS				
Number of foetuses examined	136	85	155	121
Number showing malformations	1	0	0	0
% of foetuses examined	0.7	0.0	0.0	0.0
Number showing variations	123	74	139	116
% of foetuses examined	90.4	87.1	89.7	95.9
Total number of foetuses showing malformations	1	0	0	0
% of foetuses examined	0.4	0.0	0.0	0.0

Applicant's summary and conclusion

Conclusions

Exposure to DMDS at 50 ppm elicited maternal toxicity, with associated foetal growth retardation (demonstrated by low weight and retarded ossification). There was no indication of a teratogenic effect. At 15 ppm, less marked maternal toxicity was observed and there were no foetal effects. There was no adverse effect of treatment, maternal or foetal, at 5 ppm.

Executive summary

In a developmental toxicity study, four groups of 30 mated Crl:CD(SD)BR female rats were exposed to either clean filtered or DMDS vapor atmospheres of 5, 15 or 50 ppm for 6 hours daily in whole-body inhalation chambersduring gestation days 6 through 15. All animals were observed twice daily for mortalityand moribundity. Clinical observations, body weights and food consumption were recorded at appropriate intervals. On gestation day 20, a laparohysterectomy was performed on each female. The uteri,placentaeand ovaries were examined and the pregnancy status, number of corpora lutta and the number and intrauterine position ofimplantations of live and dead fetuses and early and late resorptions were recorded. Thefetuses were weighed, sexed and examined. Approximately one half of the fetuses from each litter were dissected and the viscera examined. The carcasses were prossed for skeletal examination following staining with Alizarin red. The remaining fetuses wereplaced in Bouins solution and evaluated for soft tissue abnormalities and developmentalvariations. The study authors reported that the maternal LOAEC was 15 ppm based onreduced body weight gain. The NOAEC for maternal toxicity was reported to be 5 ppm.The fetal/developmental toxicity LOAEC, was 50 ppm based on lover mean fetal weight and retarded ossification. The NOAEC for fetal developmental toxicity was 15 ppm.

[Study 4] Barker (Hazleton-UK) 1991/K2/RF developmental toxicity-teratogenicity, rat

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report		2 (reliable with restrictions)	Barker L	1991	DIMETHYL DISULFIDE (DMDS): Inhalation range-finding study in the pregnant rat

Materials and methods

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
no guideline required		

Principles of method if other than guideline

Three groups of 7 time-mated female rats were exposed by inhalation (whole body) to concentrations of 10, 50 or 250 ppm of DMDS daily from day 6 to day 15 of gestation. A similar group of animals exposed to filtered air by the same route and over the same period acted as controls. All animals were maintained to day 20 of gestation when they were killed and their uterine contents assessed.

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Purity: 99.88%

Test animals

Species : rat

Strain : Sprague-Dawley

Administration / exposure

Route of administration : inhalation

Type of inhalation exposure (if applicable) : whole body

Vehicle : unchanged (no vehicle)

Analytical verification of doses or concentrations : yes

Duration of treatment / exposure : day 6 to day 15 of gestation

Frequency of treatment : 6 h/day

Duration of test : up to gestation day 20

Doses / concentrations : 10, 50 and 250 ppm

Control animals yes, concurrent no treatment

Results and discussions

Effect levels

Endpoint	Effect type	Effect level	Based on	Basis for effect level / Remarks

NOAEC	maternal toxicity	< 10 ppm (analytical)		

Maternal toxic effects : yes

Details on maternal toxic effects

All animals survived to day 20 of gestation. Comnon clinical signs, indicative of a loss of condition, were observed at an incidence which increased with dose, in the treated groups only. Dosage-related reductions in body weight gain were apparent in all treated groups over the exposure period. Dosage-related reductions in food intake were apparent in all treated groups over the exposure period. In the intermediate and high dose groups the lower intake persisted until termination. Water intake of the intermediate and high dose groups showed dosage-related increases from day 10 of gestation onwards, having been comparable or lower than control at the start of treatment. Water intake of the low dose group was comparable to controls throughout the study.

Embryotoxic / teratogenic effects : no effects

Details on embryotoxic / teratogenic effects

Pregnancy incidence was within the expected range in all groups. Variations in the number of corpora lutea and implantations were considered not to be an effect of treatment. Pre-implantation loss was within the expected range in all treated groups. There was no adverse effect of treatment on the incidence of intrauterine deaths. Litter size was within the expected range in all treated groups. Sex ratio was within the expected range in all groups. Mean litter weight was higher than controls in all treated groups. Mean foetal weight showed a dosage-related reduction in the treated groups, but was considered an equivocal result as values for the control and low dose groups exceeded normal limits. No malformations were observed at external examination of foetuses and the incidence of variations did not indicate an adverse effect of treatment

3.11.3 **Other data**

[Study 1] Nemec (WIL) 2006/K1/Toxicity to reproduction: other studies.

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report		1 (reliable without restriction)	Nemec MD	2006	A lactational inhalation phased-exposure study of dimethyl disulfide in rats

Materials and methods

Type of method : in vivo

Test guideline

Qualifier	**Guideline**	**Deviations**
no guideline followed		

Principles of method if other than guideline

The objective of this study (WIL-160126) was to determine the critical period of exposure during lactation required to replicate lower pup body weight results at 5, 20 and 80 ppm in a concurrent two-generation reproductive toxicity study in rats (WIL-160122; Nemec, 2006). Although the lower mean body weights and body weight gains noted in the two-generation reproductive toxicity study were occasionally statistically significantly different from the control group, the effects did not occur in an exposurerelated manner. Therefore, the current study (WIL-160126) was conducted to determine if the lower pup body weight effects noted in the two-generation study could be reproduced under conditions of exposure of the F0 adults during discrete periods of F1 postnatal development. As a result, the maternal animals in the current study (WIL-160126) were exposed to the test article at exposure levels of 5, 20 and 80 ppm during 1 of 3 selected exposure periods (lactation days 5-12 [Subset I], 13-20 [Subset II] or 5-20 [Subset III]).

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.8%

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : female

Administration / exposure

Route of administration : inhalation: vapour

Type of inhalation exposure (if applicable) : whole body

Vehicle : unchanged (no vehicle)

Analytical verification of doses or concentrations : yes

Any other information on materials and methods incl. tables

Four groups of time-mated female Crl:CD(SD) rats were exposed to either clean filtered air or vapor atmospheres of the test article, dimethyl disulfide (DMDS), for 6 hours daily in whole-body inhalation chambers. The 36 females were further divided into 3 subsets of 12 females per subset exposed according to 1 of the following regimens: Subset I was exposed from lactation days 5-12, Subset II was exposed from lactation days 13-20 and Subset III was exposed from lactation days 5-20. Target test article exposure concentrations were 0, 5, 20 and 80 parts per million (ppm). Mean measured exposure concentrations were 0, 5.0, 20.4 and 80.2 ppm, respectively, for Subset I; 0, 5.0, 20.3 and 79.9 ppm, respectively, for Subset II and 0, 5.0, 20.3 and 80.0 ppm, respectively, for Subset III. All animals were observed twice daily for appearance and behavior. Clinical observations, body weights and food consumption were recorded for all F0 females in each subset at appropriate intervals. F1 pup clinical observations and body weights were recorded on postnatal days (PND) 1, 4, 5, 7, 9, 11, 13, 15, 17, 19 and 21. F0 females and 16 of 904 WIL-160126 Dimethyl Disulfide Arkema Inc. their pups were euthanized on lactation day 21. Necropsies were conducted on all F0 females and surviving F1 pups at the scheduled euthanasia; gross lesions were retained for possible future histopathological examination. F1 pups that were found dead prior to exposure (PND 0-4) were discarded without examination. F1 pups that were found dead or euthanized in extremis on or after PND 5 were necropsied; no tissues were preserved and the carcasses were discarded.

Results and discussions

Any other information on results incl. tables

All dams in all 3 subsets survived to the scheduled necropsies on lactation day 21. There were no exposure-related clinical or macroscopic findings noted for the maternal animals at any exposure level in Subsets I, II and III. Lower mean maternal body weight gains with reduced food consumption were observed in the 80 ppm group in Subsets I, II and III during the first week of exposure when compared to the control group. At 20 ppm, lower mean maternal body weight gain and reduced food consumption were noted only on lactation days 5-7 in Subset III; however, the body weight effect at this exposure level was not observed in Subset I or Subset II. Mean maternal body weights, body weight gains and food consumption in the 5 ppm group were unaffected by test article exposure. There were no effects on the number of pups found dead, and there were no clinical or macroscopic findings noted for the F1 pups as a result of maternal exposure at any concentration in Subsets I, II and III. There were no effects on pup body weights when maternal animals were exposed to 5, 20 or 80 ppm dimethyl disulfide during lactation days 5-12 (Subset I), lactation days 13-20 (Subset II) or lactation days 5-20 (Subset III).

Applicant's summary and conclusion

Conclusions

This study (WIL-160126) was conducted in order to determine if the pup body weight effects noted in the concurrent two-generation study (WIL-160122; Nemec, 2006) at exposure levels of 5, 20 and 80 ppm were a true reflection of toxicity, and was designed to examine whether more abbreviated exposure regimens targeted during lactation or more sustained exposure was necessary to replicate the effect on pup body weights. Based on the results of this study, no effects were noted on pup body weights when dams were exposed to dimethyl disulfide at concentrations of 5, 20 and 80 ppm for 1 week during lactation days 5-12 (Subset I) or lactation days 13-20 (Subset II), or for 2 weeks during lactation days 5-20 (Subset III). Therefore, the body weight effects noted for the F1 pups in the concurrent two-generation study (WIL-160122) at 5, 20 and 80 ppm were not replicated in the current study (WIL-160126) when abbreviated exposure regimens were targeted.

3.12 Neurotoxicity

[Study 1] Nemec (WIL) 2005/K1 KS/Acute neurotoxicity, rat

Study reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key Stduy	1 (reliable without restriction)	Nemec MD	2005	An acute neurotoxicity study of dimethyl disulfide in rats

Materials and methods

Test type : acute

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 424 (Neurotoxicity Study in Rodents)	no
according to	EPA OPPTS 870.6200 (Neurotoxicity Screening Battery)	no

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.8%

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Laboratories, Inc., Raleigh, North Carolina, USA
- Age : approximately 29 or 30 days old at receipt, 43-47 days old at the initiation of dose administration
- Weight at study initiation: 165.2 g to 227.4 g for males and from 132.7 g to 195.6 g for females
- Housing: three per cage by sex for at least 3 days to facilitate adaptation to the automatic watering system. Thereafter, all animals were housed individually in clean, stainless steel, wire-mesh cages suspended above cage-board
- Diet: PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002, ad libitum

- Water: Reverseosmosis-treated (on-site) drinking water, ad libitum
- Acclimation period: 14-day

ENVIRONMENTAL CONDITIONS
- Temperature: (21.5°C to 21.7°C
- Humidity: 44.9% to 52.2%
- Air changes (per hr): 12
- Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration : other: Whole-body inhalation exposure to vapours

Vehicle : unchanged (no vehicle)

Details on exposure

EXPOSURE METHODS
The exposures were conducted (in four replicates/sex; one replicate/day) in four 1.0-m3 stainless steel and glass whole-body exposure chambers. One chamber was dedicated for each group for the duration of the study.
EXPOSURE ATMOSPHERE GENERATION
Test article was metered at a known and constant rate from a reservoir to a glass vaporization column filled with various sized glass beads. The vaporization column was wrapped with heat tapes that were operated by an Omega temperature controller. Nitrogen was delivered to the bottom of the column using a flow meter. In these generators, the liquid test article was metered onto the heated glass beads and the resulting vapor was carried out of the column by the nitrogen flowing upward through the column. Concentrated vapors were piped from the vaporization column to the chamber inlet, where the concentration was diluted to the target level by the chamber ventilation airflow. If necessary, a portion of the test article was removed after the vaporization column by way of a siphon. The control group was exposed to clean, filtered air.

Analytical verification of doses or concentrations : yes

Details on analytical verification of doses or concentrations

Actual exposure concentrations of the test article atmospheres were determined by gas chromatography (GC). Samples of the exposure atmospheres were automatically collected at approximately 35-minute intervals using a sample loop and a computer-controlled multiposition valve. The overall mean nominal concentrations for males were 120 ppm for the 100 ppm group, 240 ppm for the 200 ppm group and 885 ppm for the 750 ppm group. The overall mean nominal concentrations for females were 127 ppm for the 100 ppm group, 242 ppm for the 200 ppm group and 918 ppm for the 750 ppm group.The overall mean analyzed concentrations were 103.1, 207.0 and 745.4 ppm for males and 102.5, 206.0 and 742.9 ppm for female for the 100, 200 and 750 ppm groups, respectively.

Duration of treatment / exposure : 6 hours

Frequency of treatment : single exposure

Doses / concentrations : 100, 200 and 750 ppm

Basis analytical conc.

No. of animals per sex per dose : 12

Control animals : yes, sham-exposed

Examinations

Observations and clinical examinations performed and frequency

CLINICAL OBSERVATIONS AND SURVIVAL
All animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity.Clinical examinations were performed once daily on all animals. All significant findings were recorded. On the days that the FOB was conducted, no additional clinical signs were recorded. In addition, all animals were observed prior to exposure and at the midpoint.
BODY WEIGHTS

Individual body weights were recorded at least weekly, beginning approximately 1 week prior to test article administration (study day -7). Body weights were also recorded during FOB procedures and prior to euthanasia.

Neurobehavioural examinations performed and frequency

FUNCTIONAL OBSERVATIONAL BATTERY (FOB)
Functional observational battery (FOB) observations were recorded for all animals prior to the initiation of dose administration, at the time of peak effect on study day 0 and on study days 7 and 14. The peak effect of dimethyl disulfide was considered to be at the end of the exposure period.All animals were observed for the following parameters as described below:
* HOME CAGE OBSERVATIONS
Posture, Convulsions/Tremors, Feces consistency, Biting, Palpebral (eyelid) closure
* HANDLING OBSERVATIONS
Ease of removal from cage, Lacrimation/Chromodacryorrhea, Piloerection, Palpebral closure, Eye prominence, Red/Crusty deposits, Ease of handling animal in hand, Salivation, Fur appearance, Respiratory rate/character, Mucous membranes/Eye/Skin color, Muscle tone
* OPEN FIELD OBSERVATIONS (EVALUATED OVER A 2-MINUTE OBSERVATION PERIOD)
Mobility, Rearing, Convulsions/Tremors, Grooming, Bizarre/Stereotypic behavior, Time to first step (seconds), Gait, Arousal, Urination/Defecation, Gait score, Backing
* SENSORY OBSERVATIONS
Approach response, Startle response, Pupil response, Forelimb extension, Air righting reflex, Touch response, Tail pinch response, Eyeblink response, Hindlimb extension, Olfactory orientation
* NEUROMUSCULAR OBSERVATIONS
Hindlimb extensor strength, Hindlimb foot splay, Grip strength-hind and forelimb, Rotarod performance.
* PHYSIOLOGICAL OBSERVATIONS
Catalepsy, Body temperature, Body weight
LOCOMOTOR ACTIVITY
Observations were recorded for all animals prior to the initiation of dose administration, at the time of peak effect on study day 0 and on study days 7 and 14. The peak effect of dimethyl disulfide was considered to be at the end of the exposure period. Locomotor activity, recorded after the completion of the FOB, was measured automatically using the San Diego Instruments, Inc., Photobeam Activity System (San Diego Instruments, Inc., San Diego, California).

Sacrifice and (histo)pathology

UNSCHEDULED DEATHS
A complete necropsy was conducted on the animal found dead. The necropsy included examination of the external surface, all orifices, and the cranial, thoracic, abdominal and pelvic cavities, including viscera.
NEUROPATHOLOGY - MACROSCOPIC EXAMINATION
On study day 15, all surviving animals were euthanized by carbon dioxide inhalation and then perfused in situ with a buffered 4.0% paraformaldehyde/1.4% glutaraldehyde solution. The central and peripheral nervous system tissues were dissected and preserved for six animals/sex in the control and 750 ppm groups. Fixed brain weight (excluding olfactory bulbs) and brain dimensions (length and width) were recorded for all animals. Any observable gross changes, abnormal coloration or lesions of the brain and spinal cord were recorded.
NEUROPATHOLOGY - MICROSCOPIC EXAMINATION
The following nerve tissues were prepared for a microscopic neuropathologic examination from six animals per sex in the control and 750 ppm groups:
Brain - olfactory bulbs, cerebral cortex (2 levels), hippocampus/dentate gyrus, basal ganglia, thalamus, hypothalamus, tectum, cerebral peduncles, central gray matter, cerebellum, pons and medulla oblongata
Spinal cord - at cervical swellings C3-C7 and at lumbar swellings T13-L4
Trigeminal ganglia/nervesaLumbar dorsal root ganglia at T13-L4(bLumbar dorsal root fibers at T13-L4(bLumbar ventral root fibers at T13-L4(bCervical dorsal root ganglia at C3-C7(bCervical dorsal root fibers at C3-C7(bCervical ventral root fibers at C3-C7(bSciatic nerves (mid-thigh region)(2)(cSciatic nerves (at sciatic notch)(2)(cSural nerves(2)(cTibial nerves(2)(cPeroneal nerves(2)(cOptic nervesaEyes(aSkeletal muscle (gastrocnemius)
Other sites (if deemed necessary)
a - Both processed and evaluated microscopically.
b - Four to six tissues collected at necropsy; two evaluated microscopically.c - One processed for microscopic examination.(2) - Two sections (one cross and one longitudinal) of the tissue were evaluated fromn the right hind leg. The tissues from the left hind leg were collected and preserved for possible future evaluation.

Statistics

Body weight, body weight change, continuous functional observational battery (FOB), ambulatory locomotor activity, and brain weight, length and width data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group. Functional observational battery parameters that yielded scalar or descriptive data were analyzed using Fisher's Exact Test.

Results and discussions

Effect levels

Endpoint	**Generation (if applicable)**	**Sex**	**Effect level**	**Based on**	**Basis for effect level / Remarks**
NOAEC (neurotoxicity)		male/female	100 ppm (analytical)		treatment related effects at 200 ppm consisting of salivation during exposure in males and females and slightly soiled fur, red deposits around the mouth and tremors in females.

Results of examinations

Clinical signs and mortality : yes

Body weight and weight gain : yes

Food consumption and compound intake (if feeding study) : not examined

Food efficiency : not examined

Water consumption and compound intake (if drinking water study) : not examined

Ophthalmoscopic examination : not examined

Biochemistry : not examined

Neurobehavioural results : yes

Gross pathology : no effects

Neuropathology : no effects

Details on results

MORTALITY

One female in the 750 ppm group died during exposure on study day 0. A cause of death could not be determined at necropsy.

CLINICAL SIGNS

The only definite test article-related clinical finding was salivation. This finding was observed at the midpoint of exposure for 0, 4, 6 and 3 males and 0, 3, 6 and 6 females in the control, 100, 200 and 750 ppm groups, respectively. Salivation did not persist to the next day.

BODY WEIGHT AND WEIGHT GAIN

Mean body weight gain in the 750 ppm group males and females was lower (statistically significant at $p<0.01$ for the males) than the control group mean for study days 0 to 7. As a result, mean body weights for study days 7 and 14 and the cumulative body weight gain (study days 0 to 14) in both sexes at 750 ppm were lower (statistically significant at $p<0.05$ or $p<0.01$ for the males) than the control group values.

FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

* HOME CAGE OBSERVATIONS

A test article-related change in palpebral closure was observed in the 750 ppm group males and females at the time of peak effect on study day 0. The numbers of males and females in the 750 ppm group with drooping eyelids (half-closed) were statistically significantly ($p<0.05$) increased when compared to the control group. The percentages of animals affected in the 750 ppm group were markedly increased when compared to the WIL FOB historical control data. In the current study, 50% of the males and 36% of the females in the 750 ppm group had drooping eyelids (half-closed), while only 4% of the males and 1% of the females in the historical control data had this finding. Similar increases were not observed in the 750 ppm group at the study day 7 and 14 evaluations.

* HANDLING OBSERVATIONS

The numbers of females in the 200 and 750 ppm groups with red deposits around the mouth at the time of peak effect

on study day 0 were statistically significantly (p<0.05) higher than the control group; this finding was attributed to the test article. In addition, a change in fur appearance (slightly soiled) was observed for one male in the 750 ppm group and for one, two and three females in the 100, 200 and 750 ppm groups, respectively. This finding was related to test article exposure.
* OPEN FIELD OBSERVATIONS
Mean grooming and urination counts in the 750 ppm group males and females were significantly (p<0.05) increased when compared to the control group at the time of peak effect on study day 0. The unusually high mean urination count in the females was at least due in part to urination being counted differently for the first two female replicates compared to the remainder of the study. On these 2 days, individual urine drops were counted without taking into account animal movement during urination. Similar increases were not observed in this group at the study day 7 and 14 evaluations. Tremors were observed for 2/12 and 2/11 females in the 200 and 750 ppm groups, respectively. Even though the differences from the control group were not statistically significant, this finding has not been observed in the WIL FOB historical control data for females. Therefore, the tremors were considered to be related to dimethyl disulfide exposure.
* SENSORY OBSERVATIONS Sensory observations were unaffected by test article administration.
* NEUROMUSCULAR OBSERVATIONS
Neuromuscular observations were unaffected by test article administration.
* PHYSIOLOGICAL OBSERVATIONS
Mean body temperatures were decreased in the 750 ppm group males and females at the time of peak effect on study day 0, despite being equal to or slightly greater than at pretest. The difference from the control group was statistically significant at p<0.05 for the females. Similar decreases were not observed in this group at the study day 7 and 14 evaluations.
* LOCOMOTOR ACTIVITY
The only test article-related effect on locomotor activity consisted of decreased mean ambulatory and total motor activity values in the 750 ppm group males and females at the time of peak effect on study day 0.
BRAIN WEIGHTS AND MEASUREMENTS
Mean brain weights and measurements were unaffected by exposure to dimethyl disulfide.
MICROSCOPIC EXAMINATION
No test article-related microscopic lesions were observed in any of the central or peripheral nervous system tissues examined from six animals/sex in the 750 ppm group.

Applicant's summary and conclusion

Conclusions

Based on the results of the FOB and locomotor activity evaluations, the following domains were affected by a single 6-hour exposure of dimethyl disulfide at 750 ppm: autonomic, CNS activity, CNS excitability and physiological. The no-observed-adverse-effect concentration (NOAEC) for systemic toxicity and neurotoxicity of a single whole body inhalation exposure of dimethyl disulfide to rats was 100 ppm.

Executive summary

An acute neurotoxicity study via the inhalation route was conducted with DMDS, the study was performed following the OECD guideline # 424. In that study, four groups of 12 male and 12 female Sprague-Dawley rats were exposed whole body for 6 hours to either clean filtered air or DMDS vapor atmospheres of 100, 200 or 750 ppm. All animals were observed twice daily for mortality and moribundity, clinical examinations were performed daily except on days when the functional observational battery (FOB) was performed and body weights were recorded weekly. FOB and locomotor activity evaluations were performed on all animals prior to the initiation of exposure, at the time of peak effect following exposure and on study days 7 and 14. Following perfusion, all animals were necropsied and brain weights and brain dimensions were measured. Neuropathologic evaluations were performed on central and peripheral nervous system tissues for control and 750 ppm groups.
There were no test article-related clinical findings in the test article-treated groups. No dimethyl disulfide-related effects were apparent between treated and control group animals on sensorimotor and neuromuscular parameters when FOB (functional observational battery) evaluations were performed at the time of peak effect on study day 0 and on study days 7 and 14. Fixed brain weights and brain dimensions for perfused animals were unaffected. No test article-related neuropathological lesions were observed upon microscopic examination of 6 animals per sex in the 750 ppm group.
Test article-related effects in the 750 ppm group included the death of one female during exposure, salivation during exposure in 3/12 males and 6/12 females and decreased mean body weight gain in both sexes for study days 0 to 7. FOB parameters that were affected were observed at the time of peak effect on study day 0 and did not persist to the study day 7 and 14 evaluations. Findings observed at 750 ppm included increased numbers of animals with a change in palpebral closure (drooping eyelids in 6/12 males and 4/12 females) during the home cage observations, slightly soiled fur (1/12 males and 3/12 females) during the handling observations, increased grooming (1.0 for males and 1.4 for

females compared to control values of 0.0 and 0.2, respectively) and urination counts (2.5 for males and 4.0 for females compared to control values of 0.4 and 0.3, respectively) during the open field observations and decreased body temperature (38.1° for both sexes compared to control values of 38.5° for males and 38.6° for females) and body weight (166.3 g for males and 138.9 g for females compared to control values of 180.2 g and 149.0 g, respectively) during the physiological observations. Findings observed in the females at 750 ppm consisted of red deposits around the mouth (5/11 females) during the handling observations and tremors (2/11 females) during the open field observations. Ambulatory and total motor activity counts over 60 minutes were reduced in both sexes at 750 ppm on study day 0 only.
Test article-related effects in the 200 ppm group consisted of salivation during exposure (6/12 males and 6/12 females) and slightly soiled fur (2/12 females), red deposits around the mouth (7/12 females) and tremors (2/12 females).
The only test article-related findings in the 100 ppm group were salivation during exposure (4/12 males and 3/12 females) and slightly soiled fur (1/12 females).
Several of the aforementioned findings (drooping eyelids, salivation, increased grooming counts, red deposits and soiled fur) were most likely a result of the slightly irritating properties of dimethyl disulfide. Therefore, these test article-related findings were not considered to be indicative of systemic toxicity.
The Lowest Observed Adverse Effet Concentration (LOAEC) was 200 ppm based on treatment related effects consisting of salivation during exposure in males and females and slightly soiled fur, red deposits around the mouth and tremors in females. The No Observed Adverse Effect Concentration (NOAEC) for systemic toxicity and neurotoxicity was 100 ppm.

[Study 2] Nemec (WIL) 2006/K1 KS/ Sub-chronic neurotoxicity, rat

Study reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key Stduy	1 (reliable without restriction)	Nemec MD	2006	A subchronic inhalation neurotoxicity study of dimethyl disulfide in rats

Materials and methods

Test type : subchronic

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 424 (Neurotoxicity Study in Rodents)	no
according to	EPA OPPTS 870.6200 (Neurotoxicity Screening Battery)	no

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.8%

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Laboratories, Inc., Raleigh, North Carolina, USA.
- Age: 31 days old at receipt
- Weight at study initiation: 179 g to 234 g for males and from 147 g to 207 g for females
- Housing: individually in clean, stainless steel, wire-mesh cages suspended above cage-board
- Diet: PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002, ad libitum
- Water: Reverse osmosis-treated (on-site) drinking water, ad libitum
- Acclimation period: 13 days

ENVIRONMENTAL CONDITIONS
- Temperature: 21.3°C to 21.5°C
- Humidity: 40.5% to 55.6%
- Air changes (per hr): 10
- Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration : other: whole-body inhalation to vapours

Vehicle : unchanged (no vehicle)

Details on exposure

EXPOSURE METHODS
Animal exposures were conducted in four 2.0-m3 stainless-steel and glass whole-body exposure chambers. One chamber was dedicated to each group for the duration of the study.
EXPOSURE ATMOSPHERE GENERATION
Vapors of the test article were generated using a bubbler type (gas washing bottle) vaporization system. The test article vapors were then directed to the exposure chamber inlet where vapor concentration was reduced to the desired level by mixing with the chamber ventilation air. The control group was exposed to clean, filtered air.

Analytical verification of doses or concentrations : yes

Details on analytical verification of doses or concentrations

Actual exposure concentrations of the test article atmospheres were determined by gas chromatography (GC). The overall mean analyzed concentrations were 5.0 ± 0.14 ppm for the 5 ppm group, 20.4 ± 0.56 ppm for the 20 ppm group and 80.0 ± 1.81 ppm for the 80 ppm group.

Duration of treatment / exposure : 13 weeks

Frequency of treatment : 6 hours per day, 7 days per week

Doses / concentrations : 5, 20 and 80 ppm

Basis analytical conc.

No. of animals per sex per dose : 12

Control animals : yes, sham-exposed

Examinations

Observations and clinical examinations performed and frequency

CLINICAL OBSERVATIONS AND SURVIVAL
All animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity. Clinical examinations were performed prior to exposure, at the midpoint of exposure and approximately 1 hour after completion of exposure.
BODY WEIGHTS
Individual body weights were recorded at least weekly, beginning 1 week prior to test article exposure (study week -1).
FOOD CONSUMPTION
Individual food consumption was recorded weekly, beginning 1 week prior to test article exposure (study week -1 to 0).

Neurobehavioural examinations performed and frequency

FUNCTIONAL OBSERVATIONAL BATTERY [FOB]

Functional observational battery (FOB) assessments were recorded for all animals prior to the initiation of test article exposure and during study weeks 3, 7 and 12. Testing was performed around the time of peak effect (at the completion of exposure).All animals were observed for the following parameters as described below:

* HOME CAGE OBSERVATIONSPosture, Convulsions/Tremors, Feces consistency, Biting, Palpebral (eyelid) closure
* HANDLING OBSERVATIONSEase of removal from cage, Lacrimation/Chromodacryorrhea, Piloerection, Palpebral closure, Eye prominence, Red/Crusty deposits, Ease of handling animal in hand, Salivation, Fur appearance, Respiratory rate/character, Mucous membranes/Eye/Skin color, Muscle tone
* OPEN FIELD OBSERVATIONS (EVALUATED OVER A 2-MINUTE OBSERVATION PERIOD)Mobility, Rearing, Convulsions/Tremors, Grooming, Bizarre/Stereotypic behavior, Time to first step (seconds), Gait, Arousal, Urination/Defecation, Gait score, Backing
* SENSORY OBSERVATIONSApproach response, Startle response, Pupil response, Forelimb extension, Air righting reflex, Touch response, Tail pinch response, Eyeblink response, Hindlimb extension, Olfactory orientation
* NEUROMUSCULAR OBSERVATIONSHindlimb extensor strength, Hindlimb foot splay, Grip strength-hind and forelimb, Rotarod performance.
* PHYSIOLOGICAL OBSERVATIONSCatalepsy, Body temperature, Body weight

LOCOMOTOR ACTIVITYLocomotor activity was assessed for all animals prior to the initiation of test article exposure and during study weeks 3, 7 and 12. Locomotor activity, recorded after the completion of the FOB, was measured automatically using the San Diego Instruments, Inc., Photobeam Activity System (San Diego Instruments, Inc., San Diego, California).

Sacrifice and (histo)pathology

NEUROPATHOLOGY

* MACROSCOPIC EXAMINATIONAt the end of the exposure period (study week 13), all animals were anesthetized by an intraperitoneal injection of sodium pentobarbital and then perfused in situ with a buffered 4% paraformaldehyde/1.4% glutaraldehyde solution. The central and peripheral nervous system tissues were dissected and preserved. Fixed brain weight (excluding olfactory bulbs) and brain dimensions (length and width) were recorded. Any observable gross changes, abnormal coloration or lesions of the brain and spinal cord were recorded. Six animals/sex/group from the control and 80 ppm groups were selected for microscopic neuropathology examinations. After the central nervous system tissues were collected, the head was collected and placed in 10% neutral-buffered formalin to preserve the nasal cavity, paranasal sinuses, oral cavity, nasopharynx, middle ear, lacrimal glands and Zymbal's glands.

SLIDE PREPARATION AND MICROSCOPIC EXAMINATION

The following nervous system tissues were prepared for microscopic neuropathologic examination from 6 animals/sex in the control and 80 ppm groups:Brain - olfactory bulbs, cerebral cortex (2 levels), hippocampus/dentate gyrus, basal ganglia, thalamus, hypothalamus, tectum, cerebral peduncles, central gray matter, cerebellum, pons and medulla oblongataSpinal cord - at cervical swellings C3-C7 and at lumbar swellings T13-L4Trigeminal ganglia/nerves (aLumbar dorsal root ganglia at T13-L4

*Lumbar dorsal root fibers at T13-L4

*Lumbar ventral root fibers at T13-L4

*Cervical dorsal root ganglia at C3-C7*Cervical dorsal root fibers at C3-C7*Cervical ventral root fibers at C3-C7

*Sciatic nerves (mid-thigh region) (2)+Sciatic nerves (at sciatic notch) (2)+Sural nerves (2)+Tibial nerves (2)+Peroneal nerves (2)+Optic nervesaEyes (aSkeletal muscle - gastrocnemiusOther sites - if deemed necessary a) Both tissues were processed and evaluated microscopically.

* 4-6 tissues were collected following euthanasia; 2 were evaluatedmicroscopically.+ 1 nerve processed for microscopic examination.(2) 2 sections (1 cross and 1 longitudinal) of the tissue were evaluated from the right hind leg. The tissues from the left hind leg were collected andpreserved for possible future evaluation.

NASAL PATHOLOGY

* MACROSCOPIC EXAMINATION

The heads were collected from 6 animals/sex/group (not selected for neuropathological evaluation) from all groups and placed into 10% neutral-buffered formalin to preserve the nasal cavity, paranasal sinuses, oral cavity, nasopharynx, middle ear, lacrimal glands and Zymbal's glands.

* MICROSCOPIC EXAMINATION

The nasal tissues were prepared for qualitative histopathological examination by embedding in paraffin, sectioning and staining with hematoxylin and eosin. The nasal tissues (Levels I, II, III and IV) were prepared and evaluated according to Young, 1981.

Statistics

Body weight, body weight change, food consumption, continuous functional observational battery (FOB), ambulatory locomotor activity, and brain weight, length and width data were subjected to a parametric 1-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group.Functional observational battery parameters that yielded scalar or descriptive data were analyzed using Fisher's Exact Test. All RANOVA statistical analyses for total locomotor activity counts were conducted by BioSTAT Consultants, Inc. using SAS version 8.2 software (SAS Institute, Inc., 1999- 2001).

Results and discussions

Effect levels

Endpoint	**Generation (if applicable)**	**Sex**	**Effect level**	**Based on**	**Basis for effect level / Remarks**
NOAEC (systemic toxicity and neurotoxicity)		male	20 ppm (analytical)		Lower body weight gains, lower food consumption and lower total session motor activity counts at 80 ppm.
NOAEC (systemic toxicity and neurotoxicity)		female	80 ppm (analytical)		No adverse effect at 80 ppm.
NOAEC (local contact (nasal) irritation)		male/female	5 ppm (analytical)		Mild to moderate degeneration of the nasal olfactory epithelium at 20 and 80 ppm.

Results of examinations

Clinical signs and mortality : no effects

Body weight and weight gain : yes

Food consumption and compound intake (if feeding study) : yes

Food efficiency : not examined

Water consumption and compound intake (if drinking water study) : not examined

Ophthalmoscopic examination : not examined

Biochemistry : not examined

Neurobehavioural results : no effects

Gross pathology : no effects

Neuropathology : no effects

Details on results

CLINICAL OBSERVATIONS AND SURVIVAL
All animals survived to the scheduled euthanasia. There were no test article-related clinical findings during the detailed physical examinations, at the midpoint of exposure or at the time of peak effect (within 1 hour after exposure).
BODY WEIGHTS
Test article-related statistically significant ($p<0.05$ or $p<0.01$) lower mean body weight gains in the 20 and 80 ppm group males were noted during study week 0 to 1; these differences generally persisted through study week 8 for the 80 ppm group males.Consequently, statistically significantly ($p<0.01$) lower mean body weights and mean cumulative body weight gains were noted for the 80 ppm group males compared to the control group from study week 1 through the end of the exposure period. At the end of the exposure period (study week 13), the mean body weight of the 80 ppm group males was 16% lower than the control group.A statistically significant ($p<0.01$) test article-related lower mean body weight gain was also noted in the 80 ppm group females during study week 0 to 1. Lower, usually statistically significant ($p<0.05$ or $p<0.01$), mean cumulative body weight gains were observed in the 80 ppm group females throughout the remainder of the study primarily due to this lower mean body weight gain during study week 0 to 1. Mean body weights in this group were slightly although not statistically significantly lower compared to the control group throughout the study. At the end of the exposure period (study week 13), the mean body weight of the 80 ppm group females was 5% lower than the control group.

FOOD CONSUMPTION
Test article-related lower mean food consumption, evaluated as g/animal/day, was noted in the 80 ppm group males and females beginning study week 0 to 1. Lower mean food consumption persisted for the duration of the study in the males only, although not all intervals were statistically significant ($p<0.05$ or $p<0.01$). These lower mean food consumption values correlated with the lower body weight gains noted throughout the study in the males and during the first week of exposure in the females.
FUNCTIONAL OBSERVATIONAL BATTERY [FOB]
Home cage, handling, open field, sensory , neuromuscular and physiological (excepted the body weight) observation were unaffected by test article exposure.
LOCOMOTOR ACTIVITY
There were no test article-related effects on total or ambulatory motor activity counts in either sex during the study week 3 and 7 evaluations. Within session repeated measures analyses of variance were conducted across the subintervals of each test session for total activity counts, and linear trend analyses were conducted for overall interval means (representing the entire 60-minute session activity) during each test session. A test article-related significant decrease in overall total counts ($p\leq0.05$) was obtained for males in the 80 ppm group (35% reduction in total session activity) during the study week 12 evaluation compared to the mean control group values. These differences resulted from reduced (not statistically significant) activity levels throughout the 60-minute session.There were no effects on ambulatory motor activity in the 80 ppm group males during the study week 12 evaluation.There were no effects of the test article on habituation at any exposure level.
PATHOLOGY
* BRAIN WEIGHTS AND MEASUREMENTSThe increased mean brain weight relative to body weight observed in males at 80 ppm was statistically significant ($p<0.01$) but were considered to be a result of a test article-related decrease in terminal body weight. The mean absolute brain weight was not different from the control group.
MICROSCOPIC EXAMINATIONTest article-related degeneration of the olfactory epithelium on nasal Level II was observed with minimal to moderate severity in all 80 ppm and most 20 ppm group rats. The degree of degeneration on nasal Level II was more severe at the exposure level of 80 ppm. Olfactory epithelial degeneration was also found on Levels III and IV. This degeneration was characterized by a loss of the adluminal cytoplasmic layer of the sustentacular cells, typically with an overall thinning of the olfactory epithelium and disorganization of the underlying layers of olfactory neurons. The degeneration on nasal Levels III and IV occurred with a much higher incidence in the 80 ppm group. The only microscopic finding in the 5 ppm group consisted of minimal degeneration of the olfactory nasal epithelium on nasal Level III in 1/6 males. This was not considered to be test article-related because the findings occurred in a single animal, were of minimal severity and epithelial degeneration was not observed on any other nasal Levels in this animal. On Level II, degeneration of the olfactory epithelium was most prominent on the dorsal arches, while on Levels III and IV, the alteration typically affected the medial aspects of turbinates in the dorsal meatus. Selected histopathological findings are presented in Table 1.There were no changes in brain tissues (including olfactory bulbs), cranial, spinal or peripheral nerves, spinal nerve roots, eyes, or muscle associated with exposure to test article.

Further observations for developmental neurotoxicity study

Any other information on results incl. tables

Table 1: Selected Test Article-Related Histopathological Findings
Incidence and Severity of Degeneration of the Olfactory Epithelium in Nasal Levels I through IV

Exposure Level (ppm)		**Males**				**Females**			
		0	**5**	**20**	**80**	**0**	**5**	**20**	**80**
Young Nasal Level IIa		6	6	6	6	6	6	6	6
Degen, Olfactory. Epithelium		0	0	6	6	0	0	4	6
	Minimal	0	0	6	3	0	0	4	0
	Mild	0	0	0	2	0	0	0	1
	Moderate	0	0	0	1	0	0	0	5
Young Nasal Level IIIa		6	6	6	6	6	6	6	6
Degen, Olfactory. Epithelium		0	1	1	5	0	0	0	6
	Minimal	0	1	1	1	0	0	0	0
	Mild	0	0	0	2	0	0	0	4
	Moderate	0	0	0	2	0	0	0	2
Young Nasal Level IVa		6	6	6	6	6	6	6	6
Degen, Olfactory. Epithelium		0	0	1	4	0	0	0	4
	Minimal	0	0	1	1	0	0	0	2
	Mild	0	0	0	2	0	0	0	2

	Moderate	0	0	0	1	0	0	0	0

a= Number of tissues examined from each group.
Degen = Degeneration

Applicant's summary and conclusion

Conclusions

Systemic toxicity of DMDS administered by whole-body inhalation exposure to Crl:CD(SD) rats for 13 weeks was observed at an exposure level of 80 ppm in males as evidenced by lower body weight gains and lower food consumption through the first 8 weeks of the exposure period. Lower total session motor activity counts were noted in the 80 ppm group males during study week 12. Local contact (nasal) irritation was observed at exposure levels of 20 and 80 ppm in males and females as a dose-dependent mild to moderate degeneration of the nasal olfactory epithelium.

Executive summary

A subchronic neurotoxicity study via the inhalation route was conducted with dimethyl disulphide (DMDS) following the OECD guideline # 424. Four groups of 12 male and 12 female Crl:CD(SD)BR were exposed to either clean filtered or DMDS vapor atmospheres or 5, 20 or 80 ppm for 6 hours daily in whole-body inhalation chambers for 13 consecutive weeks. All animals were observed twice daily for mortality and moribundity and clinical examinations were perfortned daily. Body weights and food consumption were recorded weekly. Functional observational battery and locomotor activity assessments were evaluated prior to initiation of exposure and at the time of peak effect (one hour post exposure) during study weeks 3, 7 and 12. Brain weights and dimensions were determined and neuropathologic evaluations were performed. In addition, a microscopic examination of nasal tissues was performed.
DMDS-related, dose-dependent findings in the 20 and 80 ppm group males and females included lower food consumption and corresponding lower body weight gains during study week 0 to 1. Thereafter, body weight gain and food consumption for the 20 and 80 ppm group females and 20 ppm group males returned to levels comparable to the control group beginning during study week 1 to 2, but lower food consumption and body weight gains were sustained in the 80 ppm group males for the duration of the study. As a result, body weights of the males were reduced compared to the control group and cumulative body weight gains were reduced for the duration of the study for both sexes.
Lower total motor activity counts were observed in the 80 ppm group males during all sub-intervals of the study week 12 evaluation. Habituation patterns for these animals were unremarkable. Neuropathologic parameters affected by test article exposure included lower brain length in the males. Minimal to moderate degeneration of the olfactory epithelium on nasal Level II was observed in all 80 ppm males and females. In general, the olfactory epithelium of the females was more severely affected. Minimal to moderate degeneration of the olfactory epithelium was noted on nasal Level II in 6/6 males and 4/6 females in the 20 ppm group. Olfactory epithelial degeneration, characterized by a loss of the adluminal cytoplasmic layer of the sustentacular cells, was also found on Levels III and IV in the 80 ppm group. Minimal olfactory epithelial degeneration on Levels III and IV was noted in 1/6 males in the 20 ppm group. The only test article-related finding in the 5 ppm group consisted of minimal degeneration of the olfactory nasal epithelium on nasal Level III in 1/6 males. On Level II, degeneration of the olfactory epithelium was most noticeable on the dorsal arches, while on Levels III and IV, the alteration typically affected the medial aspects of turbinates in the dorsal meatus.
The no-observed-adverse-effect concentration (NOAEC) for systemic toxicity and neurotoxicity of DMDS via whole-body inhalation exposure to Crl:CD(SD) rats for 13 consecutive weeks was 20 ppm for males and 80 ppm for females. The NOAEL for local contact (nasal) irritation of DMDS via whole-body inhalation exposure for 13 consecutive weeks was 5 ppm for both males and females.

3.13 Immunotoxicity

[Study 1] Collins (Hazleton-UK) 1994/K1 KS/Immunotoxicity

Study reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key Stduy	1 (reliable without restriction)	Collins CJ	1994	DMDS: 90 day inhalation toxicity study in the rat with a 4 week recovery period

Materials and methods

Test type : subchronic

Limit test : no

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

This study was conducted to determine the inhalation immunotoxicity of Dimethyl disulfide (DMDS) in 3 treated and one control groups of male rats following administration 6 hours/day, 5 days/week over a thirteen-week period and followed by a four-week recovery period . The animals used formed part of a general toxicity study reported in IUCLID section 7.5.3 (Collins, 1992).

GLP compliance : yes (for components of the study conducted at Hazelton UK and BIBRA.)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Purity: 99.88%

Test animals

Species : rat

Strain : Sprague-Dawley

Sex : male

Details on test animals and environmental conditions

TEST ORGANISMS:
- Source: Charles River UK Ltd., Margate
- Age at reception: 4-6 weeks
- Weight at reception: 120-140 g
- Weight at the start of the treatment: 185-256 g
- Acclimatation period: 14 days

HOUSING The animals were housed in group of 5 in suspended stainless steel cages.

FOOD and WATER
- Food: SZQC rat and Mouse Maintenance Diet No. 1 ad libitum excepted during exposure
- Water: filtered tap water, ad libitum excepted during exposure

ENVIRONMENTAL CONDITIONS
- Temperature : 19-25°C
- Relative humidity : 40-70%
- Light/dark cycle : 12h/12h
- Ventilation : 15 air changes/hour

Administration / exposure

Route of administration : other: whole-body inhalation exposure to vapour

Vehicle : unchanged (no vehicle)

Details on exposure

Five horizontal flow, recirculating exposure chambers were used. Each was made of stainless steel with perspex (Plexiglas) doors and a fan to mix the atmospheres by recirculation. The compressed air supply was from a clean, dry, filtered source. The total volume of the animals did not exceed 5% of the volume of the test chamber. The four concentrations of test article vapour were produced by passing metered flows of air through sintered glass frits immersed in separate containers of test article. The resulting outputs of vapour were introduced to the diluent air inlet

duct of each test chamber. Mixing, within the duct and recirculation system, ensured the production of homogeneous atmospheres for animal exposure. The chambers were ventilated at a rate of at least 12 air changes per hour. Air flows were monitored continuously and recorded twice hourly during exposure. The exhaust streams were purified with activated charcoal and vented to the outside of the building.

Analytical verification of doses or concentrations : yes

Details on analytical verification of doses or concentrations

* Measured concentration Samples for analysis were withdrawn from the exposure chambers twice hourly through sample lines leading from each chamber through a sampling valve into a total hydrocarbon analyser. The analysis was performed with an Analysis Automation total hydrocarbon analyser type 523 Detector with a Flame ionisation detector (FID)
* Nominal concentration The total weight of test article used and total volume of diluent air were measured for each exposure. Target / Nominal / Analytical concentrations: 0 10 / 20 / 10.17 ppm 50 / 81 / 50.25 ppm 250 / 373 / 246.59 ppmA simslin II dust monitor was used pre-dose, and during the study at week 1, 4, 8, and 12, at each exposuire levels to confirm all the test article was in a vapour phase.

Duration of treatment / exposure : 13 weeks

Frequency of treatment : 6 hours/day, 5 days/week

Doses / concentrations : 10, 50 and 250 ppm

Basis analytical conc.

No. of animals per sex per dose : 20

Control animals : yes, sham-exposed

Further details on study design

- Post-exposure recovery period in satellite groups: yes, 4 weeks

<u>**Examinations**</u>

Observations and clinical examinations performed and frequency

The animal observations (mortality, clinical condition, body weight, food consumption, et c... made in connection with the general toxicity study are presented in section 7.5.3. (Collins, 1992). The procedures associated with the inmunotoxicity study are described below.HAEMATOLOGYBlood samples were obtained for the haematological studies from the ten male main study animals in 0, 10, 50 anf 250 ppm groups at week 12 and the corresponding recovery males at week 17 for the following examinations:. total white cells . total lymphocytes . pan B-cells. pan T-cells. T helper cells. T suppressor cells

Sacrifice and pathology

MYELOGRAMS
A femoral bone marrow smear was taken from all animals at necropsy and examined by a haematologist and full myelograms performed.
ORGAN WEIGHTS:
Popliteal, submandibular and mesenteric lymph nodes, thymus.
GROSS PATHOLOGY: Yes
Reported in section 7.5.3 (Collins, 1992)
HISTOPATHOLOGY: Yes
Lymph nodes (popliteal, mesenteric and submandibular), mid colon lymphoid tissue, spleen, thymus.

Humoral immunity examinations

SERUM IMUNOGLOBULIN STUDIES
Whole blood samples were obtained from the aforementioned main study animals at necropsyl for the determination of IgG and IgM titres.

Positive control

None

Statistics

- ANOVA, Regression and Dunnett's: Immunoglobins (IgG, IgM), Mylograms, Necropsy body weight (terminal kill), Organ Weights (relative and absolute) terminal kill - Popliteal Lymph Node, Mandibular Lymph Node, Mesenteric Lymph Node, Thymus, Adrenals, Spleen, Brain (absolute only).- Kruskal Wallis, Terpstra-Jonckheere, Wilcoxon Rank Sum Test : Organ weights (relative and absolute) - terminal kill - Brain (relative only).- Unpaired t test (two-tailed) and Mann-Whitner U test: lymphocytes and lymphocytes sub-populations.- Histophatology: the statistical analysis was achieved according to the test-t In order to compare the means values betwen the control group and the treated groups and the regression coefficient analysis between the dose and the different measured values.

Results and discussions

Effect levels

Endpoint	Sex	Effect level	Based on	Basis for effect level / Remarks
NOAEC	male/female	250 ppm (analytical)		No dose responsive and no treatment-related effects on the immunologic parameters

Results of examinations

Details on results

HAEMATOLOGY

The treated groups showed lymphocyte counts at week 12 that were reduced compared with the control group (Table 1). The reduction was greatest in the low dose group where the reduction was approximately 20% and did achieve statistical significance. The reductions in absolute lymphocyte counts were primarily attributable to a specific reduction in T-suppressor cells of 20 to 40% and were inversely proportional to dose level; the differences compared with the concurrent control group were statistically significant for the low and intermediate dose group but not for the high dose group.The reduction in T-suppressor cells persisted to the end of the recovery period at week 17 (Table 2) and statistically significant differences in absolute cell counts compared with the concurrent control group were present in all treated groups. There were no other statistically significant differences.There was no evidence of any treatment-related changes in T-helper cells or B-cells at either time point.

SERUM IMMUNOGLOBULIN ANALYSIS

There were no statistically significant differences from the control group and no evidence of any treatment-related differences in serum concentrations of IgG or IgM in the samples taken at the terminal kill at week 14.

MYELOGRAMS

The only treatment-related change in the myelograms taken at necropsy was a trend towards decreased lymphocytes in the treated groups. However, the routine examination of bone marrow conducted as part of the sub-chronique toxicity study (Collins, 1992, section 7.5.3) had not revealed any unusual abnormalities.

ORGAN WEIGHTS

There were no organ weight differences except for minor changes attributable to non-specific stress or reduced body weights in the high dose group.

IMNUNOHISTOPATHOLOGY QUALITATIVE PATHOLOGY

Two cases of thymic atrophy were observed: one minimal in a high dose animal, the other moderate in a control animal.Most of the mandibular lymph nodes exhibited inflamnatory reaction revealed by various degrees of sinus histiocytosis (SH) and/or medullary plasmacytosis (MP). Hemosiderosis, generally minimal, was present in some of them. The distribution of inflanmatory reactions was as follows:

Group...............Minimal...........Moderate..... .....Marked... ....Very marked or severe
........................SH..MP.............SH..MP..............SH..MP............SH..MP
control.............2.....3...............7.....2................0....0................0....0
10 ppm............1.....0...............6.....2................3....2................0....0
50 ppm............0.....0.............10.....7................0....2................0....0
250 ppm...........2.... 0...............4.....1................3....3................0....1

Inflammatory reactions seemed to be more frequent and more severe in the treated groups compared with the control group.This inflammation may result from nasal injury associated with inhalation of the test article.In addition, in one high dose animal, a cortical and paracortical atrophy of the mandibular lymph made was observed.Inflanmatory lesions (mainly sinus histiocytosis) were also observed in the mesenteric lymph mode and had the following distribution in the

various groups:
Group........... Minimal...........Moderate...........Marked.........Very marked or severe
control.............1.......................9........................0........................0
10 ppm............0......................5........................5........................0
50 ppm............0......................e.......................2........................0
250 ppm...........6................. ....4......................0.........................0
There was no evidence of a treatment-related effect.Inflammatory reaction was also observed in the popliteal lymph nodes especially sinus histiocytosis and less frequently medullary plasmacytosis. Sinus histiocytosis had the following distribution in the various groups:
Group............Minimal............Moderate...........Marked..........Very marked or severe
control.............2........................8.......................0........................0
10 ppm............0........................6.......................4........................0
50 ppm............0........................8......................2........................0
250 ppm...........4........................6.......................0........................0
There was no evidence of a treatment-related change. Hemasiderosis of both red and white splenic pulp was a frequent finding but is commonly observed in rodent spleen. Various levels of atrophy of the white pulp were observed in some animals. Lymphoid atrophy could be best characterized in the peri-arterial lymphoid sheath and had the following distribution in the various groups:
Group............Minimal...........Moderate............Marked..........Very marked or severe
control.............1.......................1........................0........................0
10 ppm............0......................4........................0........................0
50 ppm............0......................2........................0........................0
250 ppm...........0......................8........................0........................0
The distribution suggested a treatment-related change. Mid-colonThe gut associated lymphoid tissue was missing in must of the samples.Modification of gut associated lymphoid tissue was not observed by one of the pathologists. The other observed moderate hypertrophy of gut associated lymphoid tissue in the low dose group (1/4), the intermediate group (3/5) and the high dose group (1/5). These results were considered to be insignificant.
HISTOMETRY OF SPLEEN AND STATISTICAL ANALYSIS
The histometric evaluation of total surface of spleen cross section. White pulp surface, periarteriolar lymphoid sheath surface, and marginal zone surface was performed together with the statistical analysis. Pairwise statistical comparison of the control group and treated group showed:
- no significant difference between control group and the 10 ppm group.
- significant decrease of the spleen total cross section surface in the 50 ppm group without significant peri-arterial lymphoid sheath, marginal zone, red pulp.
- significant decrease of the spleen total cross section surface. white pulp. peri-arterial lymphoid sheath and marginal zone surfaces in the 150 ppm group, without significant difference for red pulp surface.Regression analysis compared the site of the various measured components between the different groups. These results confirm the observations obtained by microscopic examination of the spleen sections and confirms that a significant atrophy of the lymphoid structure of the spleen is present in 250 ppm group. For the white pulp, the periarteriolar lymphoid sheaths and the marginal zone, the slope value of the regression line is significantly negative demonstrating a reduction in the various lymphoid compartments of the spleen in the treated groups, with a dose-effect relationship. The slope value of the regression line is almost zero for the red pulp (not statistical effect of treatment) and non-significant for the whole surface

Any other information on results incl. tables

Table 1 : Absolute white blood cell (WBC) counts, % of lymphocytes, absolute lymphocytes counts, percentage of CD4+ T-helper, CD8+ T-suppressor and SIg-kappa+ B-cells and absolute numbers of T-cells, CD4+ T-helper cells, CD8+ T-suppressor cells and SIg-kappa+ B-cells at week 12.

Mean ± sd	Control	10 ppm	50 ppm
n	10	10	10
Total WBC count (10^9/L)	13.4 ± 30.	10.9 ± 1.9	13.1 ± 3.6
% lymphocytes	86.1 ± 3.3	82.2 ±8.4	83.9 ± 10.2
Absolute lymphocyte count (10e9/L)	11.5 ± 2.7	9.1 ± 2.2*	11.0 ± 3.3
% CD4+	46.1 ±3.5	49.9 ± 4.1*	48.9 ± 3.8
% CD8+	30.1 ± 5.8	23.4 ± 3.3*	23.2 ± 4.8*
%Sig-kappa+	19.4 ± 2.9	24.2 ± 4.0**	23.1 ± 4.0*
Absolute T-cell count (10^9/L)	8.82 ± 2.16*	6.63 ± 1.66	7.89 ± 2.28
Absolute T-helper count (10^9/L)	5.31 ± 1.30	4.52 ± 1.19	5.41 ± 1.82

Absolute T-suppressor count (10^9/L)	3.51 ± 1.11	2.11 ± 0.53*	2.48 ± 0.67*	2.83 ± 0.40
Absolute B-cell count (10^9/L)	2.25 ± 0.65	2.19 ± 0.66	2.52 ± 0.84	2.10 ± 0.58

* $p < 0.05$
** $p < 0.01$

Table 2 : Absolute white blood cell (WBC) counts, % of lymphocytes, absolute lymphocytes counts, percentage of CD4+ T-helper, CD8+ T-suppressor and SIg-kappa+ B-cells and absolute numbers of T-cells, CD4+ T-helper cells, CD8+ T-suppressor cells and SIg-kappa+ B-cells at week 17.

Mean ± sd	Control	10 ppm	50 ppm
n	10	10	10
Total WBC count (10^9/L)	9.6 ± 2.6	7.4 ± 1.6*	7.8 ± 2.4
% lymphocytes	83.9 ± 6.9	83.1 ± 12.3	85.1 ± 2.4
Absolute lymphocyte count (10^9/L)	7.7 ± 2.0	6.2 ± 1.7	6.6 ± 2.1
% CD4+	42.6 ± 4.5	46.9 ± 6.2	46.3 ± 5.1*
% CD8+	21.3 ± 5.2	18.5 ± 6.1	20.4 ± 5.8
%Sig-kappa+	22.4 ± 3.3	25.9 ± 6.4	25.9 ± 4.0
Absolute T-cell count (10^9/L)	4.89 ± 1.33	4.07 ± 1.28	4.37 ± 1.35
Absolute T-helper count (10^9/L)	3.32 ± 1.21	2.96 ± 1.10	3.07 ± 1.05
Absolute T-suppressor count (10^9/L)	1.57 ± 0.31	1.11 ± 0.37*	1.30 ± 0.47*
Absolute B-cell count (10^9/L)	1.71 ± 0.43	1.59 ± 060	1.74 ± 0.69

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Overall remarks, attachments

Remarks on results including tables and figures

The immunologic assessment associated with the subchronic toxicity study was extensive. The only finding or "treatment-related effect" on the measured immunologic parameters was a reduction in lymphocytes for the DMDS exposed group at the 10ppm level. A review of the study results clearly shows that the observation of lower lymphocyte counts was not dose related. In fact, the lowest lymphocyte count was noted for the low exposure concentration of 10 ppmand this was the only value that was statistically significantly lower than control. Lymphocyte courts were also included in the subchronic phase of the study and it was concluded in that phase of the study that there was no effects on total or differential white blood cell counts at any exposure concentration. The mean results of total leukocytes, total lymphocytes and percent (%) total lymphocytes to total Ieukocytes for both phases of the study are summarized in the following tables.

Summary of total leukocyte counts

	Subchronic phase	Immunologic phase
Conc. (ppm)	Mean (S.D.)	Mean (S.D.)
0	16.2 (5.3)[a]	13.4 (3.0)
10	-	10.9 (1.9)
50	-	13.1 (3.6)
150	11.5 (3.6)	-
250	12.3 (3.6)	12.4 (2.1)

[a]absolute counts expressed as 10^9/L

Summary of total lymphocyte counts and lymphocytes as a percent of total leukocytes.

	Subchronic phase		Immunologic phase	
Conc. (ppm)	Total Lymphocytes Mean (S.D.)	Lymphocytes (%) Mean(S.D.)	Total Lymphocytes Mean (S.D.)	Lymphocytes (%) Mean (S.D.)
0	13.76(4.64)[a]	85	11.5 (2.7)	86.1 (3.3)
10	-	-	9.1 (2.2)	82.2 (8.4)
50	-	-	11.0 (3.3)	83.9 (10)
150	9.90 (3.21)	86	-	-
250	10.07 (3.32)	182	10.5 (1.8)	84.8 (4.0)

[a]absolute counts expressed as 10^9/L
Charles River Laboratories (M.L.A. Giknis and C.B. Clifford, Clinical Laboratory Parameters forCrl:CD(SD)Rats, March 2006) has reported historical control mean values in Sprague Dawley rats for the above parameters of.
Total leukocytes: 11.13 (range of 9.78 – 12.90)
Total lymphocytes: 9.21 (range 8.10-10.65).
Total lymphocytes as a percent of total leukocytes: 83.93% (range 80.1 - 87.1%).

In general, the values for the above parameters in both phases of subchronic/immunologic study are consistent with the Charles River historical control values except for the total leukocyte counts with concurrently higher total lymphocyte values seen for the control males in both phases of the study. As a result, of these high control values, the treated groups were obviously numerically lower than control with statistical significance noted for the 10 ppm group and led In the erroneous conclusion of a treatment relationship.
When compared to the Charles River Laboratories data, the total leukocyte and total lymphocyte values for the DMDS exposed groups are consistent with these historical control values. A key value not mentioned by the study authors is the comparison of total lymphocytes to total leukocytes (% leukocytes). The values for "%lymphocytes" are comparable among all groups including the controls for both phases. It appears from the report for the subchronic study that total lymphocytes were derived from direct cell counts from a standard WBC differential count and the % lymphocytes was calculated fromcomparisonof the total lymphocytes to the total leukocytes. In the immunologic phase of the study, it appears that the total lymphocytes were not counted but were calculated by multiplying the total leukocytes by the % lymphocytes. The rationale for this practice is not clear but it is obvious that the lower "total lymphocytes" was directly influenced by the high leukocyte count in the control group even though the "% lymphocytes" was similar for all groups. It clearly appears that the lower total lymphocytes seen in the exposed groups from the immunologic phase is an aberration resulting from the highcontrol leukocyte count.
From the histopathologic standpoint, the same tissues were evaluated for both phases of the study. No findings were noted for the spleen and mandibular lymph nodes in the subchronic phase of the study. It appears that the special stains used for the immunologic phase revealed changes in these organs. It is not surprising that inflammation would be noted in the mandibular lymph nodes from the high dose males considering that the major target organ for toxicity was the local effect on the nasalolfactory and respiratory epithelium. In the absence of any effect on the extensive immunologic evaluations performed during immunologic phase, the findings in the spleen are considered nonspecific finding not related to an immunologic effect.

Applicant's summary and conclusion

Conclusions

The immunologic phase of the study suggests that lower lymphocyte counts were noted for the DMDS exposed groups. However, the finding was not dose responsive and not treatment-related as the only statistically significant difference was noted for the low exposed group. The lower lymphocytes counts appear to be result of higher than historical control value for the control group and the method used for determining the total lymphocytes. Evaluations of the percentage of lymphocytes to leukocytes showed that all DMDS exposed groups were similar to control. Based on the evaluation above, it can be concluded that DMDS is not immunotoxic.

Executive summary

The inhalation immunotoxicity of dimethyl disulfide (DMDS) was assessed in themale Sprague-Dawleyrat following exposure, 6 hours/day, 5 days/week over a thirteen week period to vapor concentrations of 0, 10, 50 or 250 ppm.The animals used formed part of a subchronic toxicity study reported separately (see section 7.5.3, Collins, 1992). Blood samples were obtained at week 12 and corresponding recovery animalsat week 17. The following parameters were evaluated for these samples: total white cells, total lymphocytes, pan B-tells, pan T-cells. T helper cells andT suppressor cells. In addition, IgG and IgM titres were determined in serum from thesesamples.Organ weights were determined for the popliteal lymph node, submandibularlymph node, mesenteric lymph node and thymus prior to fixation. Histologic evaluationswere performed on the popliteal lymph node, suhmandibular lymph node, mesenteric lymph node, mid colon lymphoid tissue, spleen and thymus following staining with hematoxylin-eosin saffron, with slow Giemsa and with immunocytochemical staining.
B and T cell analysis did not show any evidence of treatment-related changes in T-helper or B-tells. Reductions in absolute lymphocyte counts at week 12 in treated groups were primarily attributable to a specific reduction in T-suppressorcellsand were inversely proportional to dose level. The reduction in T-suppressor cells persisted to the end of the recovery period.There was no evidence of any treatment-related differences inserum concentrations of IgG or IgM in the samples taken at theweek 14 necropsy.The only treatment-related change in the myelograms taken atnecropsy was a trend towards decreased lymphocytes in thetreated groups.
There were no organ weight differences except for minorchanges attributable to non-specific stress or reduced body

weightsin the high dose group.
The immunohistopathology study of a range of lymphoid organsshowed variousinflammatorylesions in the threeexaminedlymphnodes. For mandibular nodes, theinflammatoryreaction was greaterin the high dose group compared with the control group. This difference was considered to be attributable to treatment.
Atrophy of the white pulp of the spleen wasobservedin most animals, including two controls. Nevertheless histometrydemonstrated a significant atrophy of the white pulp and marginalzone in the high dose group. The regression line of the atrophy was significantly negative indicating a dose-response.
The report concluded that the reduced lymphocyte counts even et 10 ppm wereattributable to a reduction in T-suppressor cells, which were inversely related to dose level and persisted to the end of the recovery period. This finding was confirmed by the trend to reduced Lymphocytes in the myelograms. Theimmunohistopathologyrevealed an increase in Inflammation of the mandibular lymph nodesat thehigh dose level of 250 ppmand a dosage-related increase insplenic lymphoid atrophy that were attributed to treatment. Histometry confirmed significant atrophy of the spleen white pulpand marginal Zone in the high dose group. There was no evidence ofsensitization.The immunologic phase of the study suggests that lower lymphocyte counts were noted for the DMDS exposed groups. However, the finding was not dose responsive and not treatment-related as the only statistically significant difference was noted for the low exposed group. The lower lymphocytes counts appear to be result of higher than historical control value for the control group and the method used for determining the total lymphocytes. Evaluations of the percentage of lymphocytes to leukocytes showed that all DMDS exposed groups were similar to control. Based on the evaluation above, it can be concluded that DMDS is not immunotoxic.

4 ENVIRONMENTAL HAZARDS

4.1 Stability

4.1.1 Hydrolysis

[Study 1] Li (2006) /K1 KS/ Hydrolysis

<u>Study Reference</u>

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable without restrictions)	Li F	2006	Hydrolysis of dimethyl disulfide in aqueous media

<u>Materials and methods</u>

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
according to	OECD Guideline 111 (Hydrolysis as a Function of pH)	no	Yes (incl. certificate)

Test materials
Test material used in the study equivalent to the substance identified in the C&L dossier.
Purity: 99.5%

Radiolabelling : yes

<u>Study design</u>

Analytical monitoring: yes

Details on sampling
Samples were analyzed at Day 0, 1, 2, 3, 4 and 5 for each pH solution (4, 7 and 9). At each time interval, three

untouched vials of each pH solution were removed from the water bath and analyzed. Each sample was analyzed for total radioactivity using liquid scintillation counting (LSC). In addition, the 14C-DMDS concentrations were evaluated using a high performance liquid chromatograph (HPLC) equipped with a radioactivity detector. After the analyses were completed, the sample vials for each respective pH concentration were combined for an average pH measurement and then discarded.

Details on analytical methods

Liquid Scintillation Counting

Radioactivity was determined by liquid scintillation counting (LSC) using Tri'-Carb 2300 TR Liquid Scintillation Analyzer (serial# 41 5529). Three vials at each time interval, as well as the dose solution were analyzed using LSC for the total 14C radioactivity. To minimize headspace in the sample vial, only one 10 µL (two 10 µL for pH 4 samples) from each vial was taken for LSC analysis. The average reading from the three samples was used for the calculation. Ten milliliters (10 mL) of scintillation cocktail (Insta -Gel Plus, Perkin Elmer) were added to each sample. The samples were counted for 5 minutes on the LSC, and all counts were automatically corrected for instrument background and efficiency. Results were reported in disintegration per minute (dpm).

HPLC

An HPLC equipped with a radiochemical detector was used for the DMDS concentration determination. The DMDS concentration in the sample was quantified based on the peak area of DMDS against a calibration curve generated using known concentrations of DMDS.

Buffers

- pH 4 buffer: mixed with 0.4 mL 0.1 N NaOH with 50 mL 0.1 M biphtalate, diluted to 100 mL with water.
- pH 7 buffer: mixed with 29.6 mL 0.1 mL N NaOH with 50 mL 0.1 M monopotassium phosphate, diluted to 100 mL with water.
- pH 9 buffer: mixed with 21.3 mL 0.1 N NaOH with 50 mL 0.1 M boric acid in 0.1 M KCl, diluted to 100 mL with water.

All glassware used in the buffer preparation and storage were sterilized by rinsing with acetone and drying for at least 2 hours in an oven set at 170°C.

Details on test conditions

The preliminary dose solution was prepared by diluting 0.14 mL standard 14C DMDS material (specific activity 2.667 mCi/mL) with 0.56 mL methanol. The resulting dose solution was five times more diluted than the standard 14C DMDS material. The dose solution was diluted by 100 fold. The definitive dose solution used on 3/20/2006 was prepared by diluting 0.1 mL standard 14C DMDS material with 0.4 mL methanol and further diluted 150 fold for LSC analysis. Ten microliter (10 µl) aliquots were taken in triplicate and used for LSC analysis. For each buffer, twenty-one, 2-mL amber HPLC vials were filled with 2040 µl buffer solutions such that each vial was completely filled, and no headspace remained in the vials. The vials were capped with a septum-closed cap. Each vial was dosed individually with 8 µL of the dose solution using a 25-µLl syringe by direct injection through the septum cap. Each vial was shaken by hand to mix the contents. Following the application of the test substance, three vials of each pH sample were analyzed as Day 0 samples. The rest of the sample vials for each pH buffer were placed in a beaker. The beakers were placed in a reciprocating, water bath with temperature controlled to 50 ± 0.1°C and an agitation rate at 80 shakes per minute. The plastic cover of the water bath was covered with aluminum foil to reduce the exposure to light and excess air flow. All vials used in the study were sterilized for at least 2 hours in an oven set at 170 °C. The temperature of the water bath was continuously monitored.

Duration of test : 5days

pH : 4, 7 and 9 **Temp.** 50

Initial conc. measured ca. 15 ppm

Number of replicates

Each day from D0 to D5, the analysis of three independent replicates were made for each pH (LSC + HPLC/radiodetection), making a total of 18 vials per pH for the whole study.

Positive controls: no

Negative controls: no

Statistical methods: None.

Results and discussion

Preliminary study

The preliminary study was conducted in aqueous solutions at pH 4, 7 and 9 at 50°C. 14C-DMDS was applied to the test solution at a rate of 14.91 ppm (approximately 0.0015%). This rate is less than half of the DMDS solubility in water (0.2%). The organic solvent in the test solution was approximately 0.4%. The measurements throughout the study showed that pH changed less than 1% within 5 days.

Test performance - Remarks

Globally, mass balance was between 90% and 110% in all daily samples, as requested by the OECD 111 guideline, except on one occasion at pH 7 at D5 where mass balance was slighty higher (112%) of the initial measured mass. It is unlikely that this slight deviation had significant impacts on the results. This is probably due to the multiplicity of sample analysis (3 replicates * 6 sampling days * 3 pH = 54 independent samples in total against 2 replicates * 2 sampling days * 3 pH = 12 independent samples in the preliminary experimental method described in the OECD 111 guideline, i.e 4.5 times less replicates). It is expected that the increased number of samples increases the global analytical uncertainty and thus the chance of one measurement being outside the 90%-110% interval while the sample actually is within this interval. Based on these results, the study was considered as valid.

Transformation products : no

Details on hydrolysis and appearance of transformation product(s)

DMDS is considered as hydrolytically stable in water at environmentally relevant pH and temperatures.

Total recovery of test substance (in %)

%Recovery	St. dev.	pH	Temp.	Duration
107		4	50 °C	5 d
96		7	50 °C	5 d
93		9	50 °C	5 d

Dissipation half-life of parent compound

pH	Temp.	Hydrolysis rate constant	Half-life	St. dev.	Type	Remarks (e.g. regression equation, r^2, DT90)
4	25 °C		> 1 yr			
7	25 °C		> 1 yr			
9	25 °C		> 1 yr			

Other kinetic parameters : Not relevant.

Details on results

Globally, HPLC-measured DMDS recovery was above 90% in all daily samples in all pH except on two occasions at pH 7 on day 3 (88%) and pH 9 on day 4 (89%) where recoveries were slightly lower than 90%. This is probably due to the multiplicity of sample analysis (3 independently fortified replicates * 6 sampling days * 3 pH = 54 independent samples in total against 2 replicates * 2 sampling days * 3 pH = 12 independent samples in the preliminary experimental method described in the OECD 111 guideline, i.e 4.5 times less replicates). It is expected that the increased number of samples increases the global analytical uncertainty and thus the chance of one replicate measured as being below 90% while it is actually above 90%). After 5 days incubation at pH 4, HPLC-measured recovery was 107%. After 5 days incubation at pH 7, HPLC-measured recovery was 96%. After 5 days incubation at pH 9, HPLC measured recovery was 93%. Thus, after 5 days incubation at 50°C at any pH, substance loss was below the 10% threshold. As a conclusion, DMDS is considered as hydrolytically stable at environmental pH (4-9) in water (half-life > 1 year at 25°C). No further hydrolysis investigations are required.

Any other information on results incl. tables

Sampling day	Sample	Mass balance	DMDS Concentration	Mean DMDS Concentration	Recovery

			(HPLC, ppm)	(ppm)	
0	pH 4	100%	14.52 17.05 14.61	15.41	100%
	pH 7	100%	13.77 16.00 14.52	14.78	100%
	pH 9	100%	15.36 13.52 15.45	14.77	100%
5	pH 4	92%	17.30 16.17 16.17	16.55	107%
	pH 7	112%	14.57 14.31 13.77	14.19	96%
	pH 9	92%	14.26 14.73 12.10	13.69	93%

Table 1: Mass balance and DMDS recovery by HPLC after 5 days incubation at 50°C and pH 4, 7 and 9.

Applicant's summary and conclusion

Conclusions

DMDS is considered as hydrolitically stable (half-life > 1 year) at environmentally relevant pH (4-9) and temperatures.

Executive summary

The objective of this study was to investigate the hydrolysis of dimethyl disulphide (DMDS) in buffered aqueous solutions at pH 4, 7 and 9 at a temperature of 50 °C.
A preliminary study was conducted prior to the definitive study. 14C DMDS was applied to the test solution at a rate of 14.91 ppm. Mass balance in the preliminary test was between 92% to 101% for pH 4 samples, 97% to 112% for pH 7 samples and 90% to 100% for pH 9 samples, indicating that there was no significant loss of radioactivity during the test period.
Concentration of DMDS in samples was measured by HPLC equipped with a radiochemical detector. The concentrations measured at D0 were set at 100% for both total radioactivity and DMDS concentration for each pH level. After 5 days incubation, DMDS recoveries based on Day 0 were 107% at pH 4, 96% at pH 7 and 93% at pH 9. The concentrations for all pH samples therefore showed no significant decrease over the five day interval. The data indicates that DMDS did not undergo hydrolysis at 50 °C in the pH 4, 7 and 9 aqueous solutions within the five day test period. DMDS is considered as hydrolytically stable (half-life > 1 year) at environmentally relevant pH and temperatures.

4.2 Biodegradation

4.2.1 Biodegradation in water: screening tests

[Study 1]: Thiébaud 1996/K2KS/Ready biodegradation

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	Key study	2 (reliable with restrictions)	Thiebaud H., Moncel N.	1995	Dimethyldisulfure: Détermination de la biodégradabilité facile - Essai en fioles fermées

Materials and methods

Test type : ready biodegradability

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
according to	OECD Guideline 301 D (Ready Biodegradability: Closed Bottle Test)	no	No
according to	EU Method C.4-E (Determination of the "Ready" Biodegradability - Closed Bottle Test)	no	No

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Study design

Oxygen conditions: aerobic

Inoculum or test system : no data

Details on inoculum :no data

Duration of test (contact time) :28 d

Initial test substance concentration

Initial conc.	Based on
3 mg/L	test mat.

Parameter followed for biodegradation estimation

DOC removal

Details on analytical methods: no data

Details on study design: no data

Reference substance: benzoic acid, sodium salt

Results and discussions

% Degradation of test substance

%Degr.	St. dev.	Parameter	Sampling time	Remarks
< 10		DOC removal	28 d	

BOD5 / COD results

Results with reference substance

More than 60% of benzoic acid, sodium salt were degraded after 14 days.

Applicant's summary and conclusion

Interpretation of results not readily biodegradable

Conclusions

- Difference between the extreme values of the test flasks at the end of the test was < 20 %.

- Percentage of degradation of the reference substance at day 14 was > 60 %.
- The degradation of the reference substance in the inhibition control test was > 25% during the 14 first days. It can be considered that the inoculum activity was not inhibited by the test substance.
- The loss of oxygen in the control test containing the inoculum was not above 1.5 mg/L of dissolved oxygen after 28 days.
- Residual concentration in oxygen in the test flasks was above 0.5 mg/L during all the test.

Executive summary

The aerobic biodegradation of dimethyldisulfure has been examinated during 28 days through OECD Guideline 301 D and EU Method C.4 -E. The results showed that less than 10 % of the substance is degraded after 28 days. The substance is not considered readily biodegradable. The substance is not toxic to microorganisms.

[Study 2] BASF AG 00/0835/27/1 (OECD 310) Biodegradation in water: screening tests

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable without restrictions)	BASF AG	2001	Study report Dimethylsulfid, Determination of the biodegradability in the CO2-Headspace test

Materials and methods

Test type : ready biodegradability

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
equivalent or similar to	OECD Guideline 310 (Ready Biodegradability - CO2 in Sealed Vessels (Headspace Test)		Yes

Principles of method if other than guideline : CO2-Headspace Test according to ISO 14593

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.5%

Study design

Oxygen conditions : aerobic

Inoculum or test system : activated sludge, domestic (adaptation not specified)

Details on inoculum

- Activated sludge from laboratory waste water plants fed with minicipal sewage
- Concentration of dry substance 4 mg/L
- No pre-adapted inoculum used
- Inoculum pre-aerated one day before the start of the test
- pH-value at beginning of the test: 7.2-7.6

Duration of test (contact time) : 28 d

Initial test substance concentration

Initial conc.	Based on
79 mg/L	test mat.

Parameter followed for biodegradation estimation

CO2 evolution

Details on study design

- About 79 mg/L test substance equivalent 20 mg/L TOC
- Degradation of the test substance at the end of the test (% TIC/ThIC): 50-60%
- Test temperature: 22 ± 2 °C

Reference substance : aniline

Results and discussions

% Degradation of test substance

%Degr.	St. dev.	Parameter	Sampling time	Remarks
50 — 60		CO2 evolution	28 d	

Details on results

- Duration of adaptation phase (days): 7
- Duration of degradation phase (days): 21
- Kinetic of test substance (in %):

= 8 after 7 day(s)
= 47 after 14 day(s)
= 44 after 21 day(s)
= 53 after 28 day(s)
- Degradation products: not measured

BOD5 / COD results

Results with reference substance

Kinetic of reference substance (in %):
= 71 after 7 day(s)
= 91 after 28 day(s)
- Degradation of the reference substance after 14 days (% TICThlC): 80-90%
- Degradation degree in the inhibition control 14 days (% TIC/ThlC): 40-50%

Any other information on results incl. tables

RESULTS
- Biodegradation degree [% TIC/ThIC] after 28 days: 50-60%
- Degradation degree of the test substance not above 60% within 28 days
- 10-day window not met
- the test substance is moderately or partly biodegradable under the test conditions (not readily biodegradable according to OECD criteria)

VALIDITY CRITERIA
- Max. deviation of the degradation degree of the test substance at the end of the test <20%: yes
- Degradation degree of the reference substance above 60% after 14 days: yes
- Degradation degree in the inhibition control below 25% after 14 days: yes
- TIC-value (mg/L) of the blank after 28 days below 15 % of the amount of test substance added initially (mg ThIC/L): yes
- The test is valid: yes
- Physical-chemical (abiotic) elimination of the test substance at the end of test (% TIC/ThIC) < 10%

Applicant's summary and conclusion

Moderately or partly biodegradable under the test conditions. Not readily biodegradable.

4.2.2 Degradation simulation testing in sediment

Study 1: Allan 2011/K1KS/Biodegradation in sediment: simulation test

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable without restrictions)	Allan J.	2011	14C-Dimethyl Disulfide: Aerobic Transformation in Aquatic Sediment Systems

Materials and methods

Test type : degradation simulation test

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
according to	OECD Guideline 308 (Aerobic and Anaerobic Transformation in Aquatic Sediment Systems)	Yes	Yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Study design

Oxygen conditions: aerobic

Inoculum or test system: natural water / sediment

Details on inoculum: The water/sediment systems were from Tift County, Georgia, and Audrain County, Missouri. The Tift water used in the definitive test had a pH of 9.1 while the sediment was characterized as a sand (USDA textural class) with a pH of 7.5 (1:1 soil:0.01 M CaCl2 ratio) and organic matter content of 0.70% (Walkley Black method). The Audrain water used in the definitive tests had a pH of 6.8, while the sediment was characterized as a silt loam (USDA textural class) with a pH of 5.1 (1:1 soil:0.01 M CaCl2 ratio) and organic matter content of 2.3% (Walkley Black method). Another collection of sediment/water from Audrain County was used in the preliminary test and had similar properties to that used in the definitive test.
The microbial biomass of the sediments was determined with initiation of the definitive test by fumigation/extraction analysis of the sediment:
Microbial Biomass at Initiation (µg/g dry weight) were 11.1 for Tift and 6.9 for Audrain.

Duration of test (contact time): 7 days

Initial test substance concentration

Initial conc.	Based on
50 mg/L	test mat.

Parameter followed for biodegradation estimation

Radiochemical follow-up

Details on analytical methods: Water layer: LSC for radioactivity content and then filtered and analyzed by direct

injection using reversed-phase HPLC/UV ; Sediment layer: extracted with a mixture of aqueous and organic solvents and analyzed by LSC and reversed-phase HPLC ; Volatiles traps were analyzed by LSC ; Volatile organic traps extracted and analyzed by reversed-phase HPLC.

Details on study design: For each sediment/water system, a total of 17 test vessels were prepared (one to serve as a control sample for monitoring as dissolved oxygen (DO), pH of the sediment and water, and redox potentials of the sediment and water; and 16 for replicated sampling events). These test bottles were prepared by transferring approximately 2.5 cm height of sediment to each bottle. A sufficient volume of the corresponding water was then added to the bottles to bring the total sediment/water column to 9 cm, which equates to a 7.5 cm water column and at least a 3:1 water:sediment ratio.

Reference substance: none, not required

Other information: due to potential inhalation hazards of radiolabeled test substance should the trapping system fail, the test was performed in a laboratory fume hood where laboratory light and temperature were not controlled as directly as listed in the guidelines. For temperature, the range experienced was between 18 and 24.5°C instead of 20 ± 2°C. For lighting, the test was subjected to standard laboratory light throughout the work day, which usually runs weekdays from 7 am to 8 pm. Given the results of the testing, these deviations did not adversely affect the results or their interpretation.

Results and discussions

% Degradation of test substance

%Degr.	St. dev.	Parameter	Sampling time	Remarks
< 5		Radiochemical measurement	7 h	

Test performance: A preliminary test on one water/sediment aerobic system with a duration of 10 days showed that dissipation of DMDS from the system took place very rapidly. Therefore the definitive test on the 2 water/sediment aerobic systems was designed for only a 7 hours total duration, which was sufficient to show that DMDS partitions to the atmospheric compartment is purged quantitatively from the system as parent material.

Results with reference substance: Not applicable.

Transformation products: None.

Applicant's summary and conclusion

Conclusions

This study showed that aerobic transformation in an aquatic/sediment system is not a major pathway of degradation of DMDS. Instead, the test substance dissipates from the water column. The dissipation of radiolabeled DMDS was studied in two different flooded sediment systems under aerobic conditions. Volatile DMDS dissipated from the water rapidly. Dissipation of DMDS from the overlying water to the sediment was low.

Executive summary

The biotransformation of [14C]dimethyl disulfide (DMDS) was studied in two different flooded sediment systems under aerobic conditions. The water/sediment systems were from Tift County, Georgia (USA) and Audrain County, Missouri (USA). The Tift water had a pH of 9.1, while the sediment was characterized as a sand (USDA textural class) with a pH of 7.5 (1:1 soil:0.01 M $CaCl_2$ ratio) and organic matter content of 0.70% (Walkley Black method). The Audrain water had a pH of 6.8, while the sediment was characterized as a silt loam (USDA textural class) with a pH of 5.1 (1:1 soil:0.01 M $CaCl_2$ ratio) and organic matter content of 2.3% (Walkley Black method).
The water phase of the test system was treated with [14C]-DMDS at a concentration of 50 µg/g water. Due to potential safety hazards, the test systems were incubated in a fume hood at room temperature. Aerobic conditions were maintained by passing a steady stream of humidified air through the test apparatus. The flow-through systems were designed to trap evolved carbon dioxide (CO_2) and volatile organic compounds. Samples were taken at 0, 1, 2, 3, 4, 5, 6, and 7 hours after application. The water and sediment layers were separated by decanting. The water layer was analyzed by LSC for radioactivity content and then filtered and analyzed by direct injection using reversed-phase HPLC. The sediment layer was extracted with a mixture of aqueous and organic solvents and analyzed by LSC and reversed-phase HPLC. Volatiles traps were analyzed by LSC, and the volatile organic traps were extracted and analyzed by reversed-phase HPLC, too.

No major degradation product, which is defined as =10% AR or at =5% AR at two consecutive sampling intervals, was observed during the course of the study in any of the matrices. No significant radioactivity (=5% AR) was found in the sediment layer as extractable or non-extractable residues (NER). The amount in the water phase quickly dissipated out of the water phase and into the organic volatile traps, where it was captured as parent DMDS. The rate of dissipation of DMDS from the water/sediment systems was directly proportional to the flow rate of the flow of air through the test systems, which was needed to maintain the desired environmental conditions. However, the radioactivity captured was still parent DMDS, and thus aerobic transformation is not a pathway of degradation in a water/sediment system as indicated by the study results. This result was anticipated since the test substance is a fumigant.

4.2.3 Degradation simulation testing in soil

[Study 1]: Conway 2007/K1KS/Biodegradation in soil: simulation test

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable without restrictions)	Conway S.	2007	Aerobic soil metabolism of Dimethyldisulfide

Materials and methods

Test type : degradation simulation test

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
according to	EPA OPPTS 835.3300 (Soil Biodegradation)	No	Yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Study design

Oxygen conditions: aerobic

Soil classification: USDA

Details on soil characteristics: Soils were freshly sampled from representative suitable agricultural sites. All samples were taken from plots not treated by pesticide or fertilizer for at least 5 years prior to sampling. The moisture content of each soil was adjusted to approximately 40% of the maximum water content using Millipore deionized water, and incubated at 20°C in the dark and in contact contact with the air. Each soil was sieved though a 2 mm sieve prior to use.
Test soils: #1 San Luis Obispo, CA, USA (CA), loamy sand ; #2 Quincy, FL, USA (FL), sand ; #3 Itingen-AT, Switzerland (CH), clay ; #4 Ruelisheim, France (FR), clay loam

Duration of test (contact time): 120 days

Initial test substance concentration

Initial conc.	Based on
900 kg/ha d.w.	test mat.

Parameter followed for biodegradation estimation

Radiochemical follow-up and CO2 evolution

Details on analytical methods:

Measurement of radioactivity

Liquid extracts
The quantity of radioactivity in liquid extracts was determined by a Packard liquid scintillation counter equipped with DPM and luminescence options. Insta-Gel Plus Universal Scientillation Coktail was used. The efficiency of hte counter was checked once a day by measuremnt of a Packard-vial containing a known amount of radioactivity. All measurements were performed at least in duplicate, corrected for background and counted for a time interval that provided a counting error below 5%.

Soils
Wet soil sample were homogenized by vigorous shaking for 10 min and a representative sample of each was transferred to a small glass oxidizer boat. The sample was then heated to 900°C under an oxygen atmosphere in a biological oxidizer. The instrument converts all organic carbon in the sample to carbon dioxide, which is captured in a mixture of liquid scintillation cocktail and potassium hydroxide. The efficiency of the instrument was verified before and after each sample set by combustion of a known quantity of 14C-labeled mannitol. The combustion efficiency remained consistently greater than 90%.

Carbon dioxide
Radioactivity was determined in the 1N KOH tube contained within the test system. A second 0.5N KOH trap positioned after the charcoal tube contained negligible radioactivity. For precipitation of the carbon dioxide, a barium carbonate precipitation method was used. A 1.0 ml aliquot of 1M $BaCl_2$ solution was added to a 1.0 ml aliquot from each KOH trap which had been transferred to a 10 ml plastic centrifuge tube. The samples were vortexed for approximately 10 sec., left to stand, and than centrifuged at 3000 rpm for approximately 2 min. A 100 µl aliquot of hte supernatant was transferred to a scintillation cocktail and counted. The entire supernatant was removed and the precipitate was added to scintillation cocktail along with 100 µl of a 1N HCl solution and counted.

Experimental conditions

Tests were carried out at 20°C. Humidity was 19.1, 23.5, 73.8 and 66.02% for soils 1, 2, 3 and 4 respectively. Microbial biomass was 86, 99, 474 and 352 for soils 1, 2, 3 and 4 respectively.

Results and discussions

Mass balance: between 93.6% and 102.8% recovery in all four soils.

% Degradation of test substance

Soil	%Degr.	Parameter	Sampling time	Remarks
1	12.3	Sum of KOH, various soil extracts and PES	120 d	
2	15.4	Sum of KOH, various soil extracts and PES	120 d	
3	42.7	Sum of KOH, various soil extracts and PES	120 d	
4	41.9	Sum of KOH, various soil extracts and PES	59 d	

DT50 of parent compound

Soil	DT50	Type	Temp	Remarks
1	2.8 d	(pseudo-)first order (= DT50)	20.0°C	
2	2.8 d	(pseudo-)first order (= DT50)	20.0°C	
3	3.0 d	(pseudo-)first order (= DT50)	20.0°C	
4	2.7 d	(pseudo-)first order (= DT50)	20.0°C	

Transformation products: yes, carbon dioxide (CAS 124-38-9) and methanesulfonic acid (CAS 75-75-2)
Evaporation of parent compound: yes
Volatile metabolites: yes
Residues: yes

Applicant's summary and conclusion

Conclusions

This study showed that aerobic transformation in a soil system is not a major pathway of degradation of DMDS. Instead, the test substance dissipates from the soil rapidly. This study gives further credence to the fact that DMDS rapidly dissipates in the soil due to volatilization and that this is likely the primary dissipation mechanism occurring in the soil.

Executive summary

The aerobic degradation of DMDS was studied in two light soil, a California loamy sans and a Florida sand, representative of locations typically associated with fumigation and with two heavier European soils, a French clay loam and a Swiss clay.

Preliminary work during the methods development phase conducted with DMDS indicated that aerobic soil degradation could not be determined through a standard flow-through test system. Concerns about maintaining an aerobic soil environment during the course of the study resulted in the development of a unique test system related to the propensity for DMDS to leak through various inert materials. For this reason, a test system with a minimum of connections and Teflon seals was required to maintain the mass balance.

Prior to conducting the study, the moisture content of each soil was adjusted to approximately 40% of the maximum water holding capacity, and the soil was acclimated by exposure to the air at 20°C prior to use. Soil samples were then sieved (2 mm). Soil was placed into the 120x55 mm, 250 mL, neck 17 mm diameter, side-arm 1mm ID flask that served as test system. A 160x100 mm borosilicate glass culture tube containing 1N potassium hydroxide solution was inserted into each sample bottle to absorb any carbon dioxide released from the test system. The soil was treated with a solution of 14C-labeled DMDS in ethanol. Once prepared, the samples were fully mixed and incubated at 20°C in the dark for up to 120 days.

Duplicate sample bottles were removed at intervals for analysis. At sampling, the headspace of each bottle was thoroughly swept with air through a two-stage SKC Anasorb CSC (coconut shell charcoal) tube. For the heavier soils, upon analysis, the charcoal tubes were removed, extracted with hexane, and analyzed by LSC and HPLC with radiochemical and ultraviolet spectrophotometric detection. The KOH solution was removed from the test system and analyzed by LSC, by precipitation of CO2 with reagent and by HPLC. The soil was extracted sequentially with acetonitrile, water and sodium hypochlorite solutions. Each extract was analyzed separately by LSC and HPLC to measure organic-soluble, water-soluble and oxidizable chemical secies, respectively. Finally, the extracted soil was combusted under oxidative conditions to determine the amount of soil-bound radioactive components.

The study results demonstrate that the primary metabolic products of DMDS in soil under aerobic conditions are carbon dioxide and methanesulfonic acid. No other radioactive species were present at greater than 10% of the applied dosage. Concentrations of methanesulfonic acid greater than 25% were detected in the Swiss and French soils after 7 days of incubation and low though significant amounts were detected in the California and Florida soils by the end of the study. The highest level of carbon dioxide was detected in the final sampling point for the Swiss soil with a yield of 6.72% of the total dose.

Literature studies referenced within this report indicate that methanesulfonic acid can be degraded in soil to formaldehyde, which can then enter the C1 metabolic cycle. These natural carbon-based constituents were not identified in this study, presumably because the concentrations remained low and the subsequent rate of mineralization to carbon dioxide was sufficiently rapid. Another degradate of DMDS, sulfurous acid, is also postulated. This compound in turn would oxidize to sulfuric acid. However, the radiochemical study was not designed to detect these acids.

Although the test system was carefully designed to retard volatilization and loss from the test system, this process still remained a key factor within the study. Due to a lack of appropriate and standardized computer program for making correction for volatilization, DT50s presented in this summary include loss due to both volatilization and degradation. This study gives further credence to the fact that DMDS rapidly dissipates in the soil due to volatilization and that this is likely the primary dissipation mechanism occurring in the soil.

4.3 Bioaccumulation

4.3.1 Bioaccumulation: aquatic / sediment

Information related to DMDS partition coefficient indicates that the substance has a low potential for bioaccumulation based on a measured log Kow of 1.91. Moreover due to its high volatility potential, DMDS is not expected to persist in water column. Therefore taking into consideration these two characteristics, bioaccumulation test on aquatic species is deemed scientifically unjustified.

[Study 1] Diepenhorst 2006/K1 KS/Partition coefficient

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable without restrictions)	Diepenhorst PC	2006	Relative density, solubility in organic solvents and partition coefficient Pow of DMDS

Materials and methods

Partition coefficient type : octanol-water

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
according to	OECD Guideline 107 (Partition Coefficient (n-octanol / water), Shake Flask Method)		yes (incl. certificate)

Type of method : shake-flask method

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Details on methods

The temperature of the laboratory room for the sample preparation varied during the manipulation between 20.4°C and 20.9°C.The pH of the water layers in the test varied between 6.61 and 6.95.

Results and discussions

Partition coefficient

Type	Partition coefficient	Temp.	pH	Remarks
log Pow	1.91	20.6 °C	ca. 6.7	

Details on results

The mean octanol/water partition coefficient Pow is 1.81 with a standard deviation of 0.22. The resulting log pow is 1.91.

Applicant's summary and conclusion

Log Kow of dimethyl disulfide has been investigated throught OECD guideline 107 (shake flask method).
The mean octanol/water partition coefficient Pow was 1.81 with a standard deviation of 0.22, resulting in log Kow of 1.91 at 20°C.

4.4 Acute toxicity

4.4.1 Short-term toxicity to fish

[Study 1] rainbow trout, Scheerbaum 2007/K1 KS/Acute fish

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable without restrictions)	Scheerbaum D.	2007	DIMETHYL DISULFIDE TC: Fish (Rainbow trout), acute toxicity test, semi-static, 96h

Materials and methods

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
according to	other guideline: EPA OPPTS Draft Guideline 850.1075 (1996)	no	yes (incl. certificate)

Test materials
Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.89%

Test method

Analytical monitoring : yes

Details on sampling

Aliquots of the samples were diluted in 20 mL headspace vials pre-filled with aqueous sodium chloride or 0.1 g NaCl for 0.625 mg/L and control.

Details on analytical methods

- Separation method: GC
- Detection method: Mass Selective (MS)
- LOQ: 10.0 µg DMDS/L
- Precision: mean recovery rates should be between 70 and 110 %. Relative standard deviation should be lower than 20 %.
- Specificity: 2 blank samples were used to prove specificity and blank values being < 30 % of the LOQ.

Vehicle : yes

Details on test solutions

A stock solution of the standard of 1000 mg/L was prepared in methanol. Dilutions were prepared in methanol. 0.1 mL of each dilution was diluted with 9.9 mL aqueous sodium chloride (10 g/L).

Test organisms

Test organisms (species) : Oncorhynchus mykiss

Details on test organisms

- Source: Forellenzucht trostadt GbR
- Feeding during test
- Food type: SGP 493 (Krystal) Export
- Amount: 4% of the fish body weight per feeding day
- Frequency: daily
- Length at study initiation: 4.60 cm
- Weight at study initiation: 0.68 g
- Holding: 12 ± 2ºC, diffuse light, under flow-through conditions, water exchange 1-2 times the aquarium volume per day, dissolved oxygen concentration more than 80% of the air saturation value.
- Rainbow trout with at least 12 days of acclimatisation and mortality <5% within these days used for the test
- No disease treatments administered throughout holding and testing

Study design

Test type : semi-static

Water media type : freshwater

Limit test : no

Total exposure duration : 96 h

Test conditions

Hardness : 10 - 250 mg CaCO3/L

Test temperature : 12 ± 1ºC

pH : 6.0 - 8.5

Dissolved oxygen:

Not less than 60% of air saturation value

Salinity : no data

Nominal and measured concentrations

Nominal concentrations: 0.625 - 1.25 - 2.5 - 5 and 10 mg/L
Measured concentrations: 0.227 - 0.541 - 1.26 - 3.07 and 6.90 mg/L

Details on test conditions

TEST SYSTEM
- Test vessel: Glass-aquaria (total volume of 23 L), loosely covered by glass tops
- Test volume: 21 L per vessel
- No aeration
- No. of organisms per concentration: 10
- Density in the tanks less than 0.8 g fish per liter test
- 1 replicate per test concentration and control
- Fish added to the vessel at least 30 min after the addition of the test item to the test water

TEST MEDIUM / WATER PARAMETERS
- Source of water: tap water of local origin used for holding and testing; water filtered on activated charcoal and aerated for at least 24h to remove chlorine.
- Residual chlorine: <0.003 mg/L

OTHER TEST CONDITIONS
- No feeding during test
- Photoperiod: 12h light:12h dark, with 15-30 min transition period
- Light intensity: 0.1 - 10 µmol photons/m²/s

Reference substance (positive control) : not required

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	0.97 mg/L	meas. (geom. mean)	test mat.	mortality	95% CL: 0.96 - 0.98 mg/L
96 h	NOEC	0.541 mg/L	meas. (geom. mean)	test mat.	mortality	

Details on results

Due to the high volatility losses of the test item occured, all effect levels are based on geometric mean measured concentrations.

Results with reference substance (positive control) : not applicable

Applicant's summary and conclusion

Conclusions

Validity criteria:
- O2 saturation > 60 %
- Mortality in the control < 1/10 fish

Executive summary

Acute toxicity to Rainbow trout (Oncorhynchus mykiss) was investigated through EPA OPPTS Draft Guideline 850.1075 (1996). Oncorhynchus mykiss juveniles were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 0.625 - 1.25 - 2.5 - 5 and 10 mg/L.
96h-LC50 was 0.97 mg/L and 96h-NOEC was 0.541 mg/L.

[Study 2]: zebra fish, Scheerbaum 2007/K1/Acute fish

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	supporting study	1 (reliable without restrictions)	Scheerbaum D.	2007	DIMETHYL DISULFIDE TC: Fish (Zebrafish), acute toxicity test, semi-static, 96h

Materials and methods

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
according to	other guideline: EPA OPPTS Draft Guideline 850.1075 (1996)	no	yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.89%

Test method

Analytical monitoring: yes

Details on sampling

Aliquots of the samples were diluted in 20 mL headspace vials pre-filled with aqueous sodium chloride.

Details on analytical methods

- Separation method: GC
- Detection method: Mass Selective (MS)
- LOQ: 10.0 µg DMDS/L
- Precision: mean recovery rates should be between 70 and 110 %. Relative standard deviation should be lower than 20 %.
- Specificity: 2 blank samples were used to prove specificity and blank values being < 30 % of the LOQ.

Vehicle : yes

Details on test solutions

A stock solution of the standard of 1000 mg/L was prepared in methanol. Dilutions were prepared in methanol. 0.1 mL of each dilution was diluted with 9.9 mL aqueous sodium chloride (10 g/L).

Test organisms

Test organisms (species) : Danio rerio

Details on test organisms

- Source: Aquarium Am Aegi, Hannover
- Feeding during test
- Food type: Stör perlets, Heinsberg; not fed 24h before test start
- Amount: 4% of the fish body weight per feeding day
- Frequency: 3 times per week
- Length at study initiation: 2.59 cm
- Weight at study initiation: 0.23 g
- Holding: 23 ± 2ºC, diffuse light, water changed at least once per week, dissolved oxygen concentration more than 80% of the air saturation value
- Zebrafish with 12 days of acclimatisation and mortality <5% within these days used for the test
- No disease treatments administered throughout holding and testing

Study design

Test type: semi-static

Water media type : freshwater

Limit test : no

Total exposure duration : 96 h

Test conditions

Hardness : 10 - 250 mg CaCO3/L

Test temperature : 20 - 24 ± 1ºC

pH : 6.0 - 8.5

Dissolved oxygen : Not less than 60% of air saturation value

Salinity : no data

Nominal and measured concentrations

Nominal concentrations: 6.25 - 12.5 - 25 - 50 and 100 mg/L
Measured concentrations: 3.3 - 7.59 - 19.0 - 33.4 and 71.0 mg/L

Details on test conditions

TEST SYSTEM
- Test vessel: Glass-aquaria (total volume of 14.5 L), loosely covered by glass top
- Test volume: 13.5 L per vessel
- No aeration
- No. of organisms per concentration: 10
- Density in the tanks less than 0.8 g fish per liter test
- 1 replicate per test concentration and control
- Fish added to the vessel at least 30 min after the addition of the test item to the test water

TEST MEDIUM / WATER PARAMETERS
- Source of water: tap water of local origin used for holding and testing; water filtered on activated charcoal and aerated for at least 24h to remove chlorine.
- Residual chlorine: <0.003 mg/L

OTHER TEST CONDITIONS
- No feeding during test
- Photoperiod: 12-16h illumination with 15-30 min transition period
- Light intensity: 0.1 - 10 µmol photons/m²/s

Reference substance (positive control) : not required

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	5.01 mg/L	meas. (geom. mean)	test mat.	mortality	95% CL: 3.30 - 7.59 mg/L
96 h	NOEC	3.3 mg/L	meas. (geom. mean)	test mat.	mortality	

Details on results

Due to the high volatility losses of the test item occured, all effect levels are based on geometric mean measured concentrations.

Results with reference substance (positive control) : not applicable

Applicant's summary and conclusion

Conclusions

Validity criteria:
- O2 saturation > 60 %
- Mortality in the control < 1/10 fish

Deviations from the guideline: none

Deviations from the study plan: for the determination of the body weight and length, 7 fish instead of 10 were used. The confidence interval after 48, 72 and 96h was not calculated, since only concentrations with no mortality or complete mortality were observed. These deviations did not affect the results and the outcome of the study.

Executive summary

Acute toxicity to Zebrafish (Danio rerio) has been investigated through EPA OPPTS Draft Guideline 850.1075 (1996). Danio rerio were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 6.25 - 12.5 - 25 - 50 and 100 mg/L. 96h-LC50 was 5.01 mg/L and 96h-NOEC was 3.3 mg/L.

[Study 3] Sheepshead minnow, Minderhout 2008/K1/Acute fish

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	supporting study	1 (reliable without restrictions)	Minderhout T., Kendall T.Z., Krueger H.O	2008	DIMETHYL DISULFIDE: A 96-HOUR STATIC-RENEWAL ACUTE TOXICITY TEST WITH THE SHEEPSHEAD MINNOW (Cyprinodon variegatus)

Materials and methods

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
according to	EPA OPPTS 850.1075 (Freshwater and Saltwater Fish Acute Toxicity Test)		yes (incl. certificate)
according to	other guideline: U.S. Environmental Protection Agency, Standard Evaluation Procedure, Acute Toxicity Test for Estuarine and Marine		

	Organisms		
according to	other guideline: ASTM Standard E729-96: Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians		

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.6%

Analytical monitoring : yes

Details on sampling
Water filtered and diluted to a salinity of approximately 20‰ with well water.

Details on analytical methods
- Separation method: HPLC with ultraviolet detection
- Chromatographic separations were achieved using a YMC-Pack ODS-AM column (150 mm x 4.6 mm, 3-µm particle size)
- Limit of quantitation (LOQ) was defined as 2.00 mg a.i./L, calculated as the product of the concentration of the lowest calibration standard (2.00 mg a.i./L) and the dilution factor of the matrix blank samples (1.00).

Vehicle : yes

Details on test solutions
Individual test solutions were prepared for the daily renewal in the approximately 13.5-L glass water accommodated fraction (WAF) bottles at nominal concentrations of 2.6, 4.3, 7.2, 12 and 20 mg a.i./L. The calculated amount of test substance were measured and transferred into WAF bottles containing filtered saltwater and Teflon®-lined magnetic stir bars. The bottles were completely filled to minimize headspace and then capped with silicone stoppers. The solutions were stirred for approximately 24 hours. After mixing, two replicates of each test solution were prepared by decanting approximately 3.8 L of test solutions per test chamber. During the test, the new test solutions were prepared daily in separate test chambers, and all surviving organisms were transferred from old to new solutions at approximately 24-hour intervals. All test solutions were adjusted to 100% active ingredient during preparation, based on the test substance purity (99.6%) and corrected for the specific gravity of 1.063. All test solutions appeared clear and colorless at test initiation and termination.

Test organisms

Test organisms (species) : Cyprinodon variegatus

Details on test organisms
- Source: Aquatic Bio Systems, Fort Collins, Colorado
- Age: juveniles
- Mean wet weight: 0.10 g (range: 0.08 – 0.15 g)
- Mean total length: 2.0 cm (range: 1.8 – 2.2 cm)
- Held for at least 14 days prior to the test in water from the same source as the test and at approximately the same temperature as used during the test. Dissolved oxygen concentrations ranged from 7.1 to 7.6 mg/L (≥91% of saturation) and salinity of the water ranged from 20 to 21 parts per thousand.
- Food: commercially-prepared diet during the holding period; not fed for at least two days prior to the test initiation and during the test.
- No signs of disease or stress during the 2-week period prior to the test.

Study design

Test type : semi-static

Water media type : freshwater

Limit test : no

Total exposure duration : 96 h

Test conditions

Hardness : There were no data on water hardness. Hardness is not expected to affect non-acidic substance toxicity.

Test temperature : Temperature was 22 ± 2ºC.

pH : pH ranged from 8.0 to 8.2

Dissolved oxygen : Dissolved oxygen remained ≥5.3 mg/L (68% of saturation) throughout the test.

Salinity : no data

Nominal and measured concentrations

Nominal concentrations: 2.6 - 4.3 - 7.2 - 12 and 20 mg active ingredient (a.i.)/L
Measured concentrations: 2.3 - 4.0 - 6.9 - 10 and 18 mg active ingredient (a.i.)/L

Details on test conditions

TEST MEDIUM / WATER PARAMETERS
- 10 sheepshead minnows per test chamber
- Total of 20 fish per test concentration
- Closed-bottle test chambers with minimal headspace were used
- Test solutions were renewed at approximately 24-hour intervals
- Fluorescent light bulbs that emit wavelengths similar to natural sunlight were used for illumination of the cultures and test chambers
- Photoperiod of 16 hours of light and 8 hours of darkness

TEST SYSTEM
- Test chambers: 3.8-L glass jars with Teflon®-lined lids containing approximately 3.8 L of test solution.
- Depth of the test water in a representative test chamber was approximately 24 cm.

Reference substance (positive control) : no data

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	LC50	5.6 mg/L		test mat.	mortality	
96 h	NOEC	< 2.3 mg/L		test mat.		

Details on results

All sheepshead minnows in the negative control group appeared healthy and normal throughout the test. No mortality was observed at test termination in the 2.3 and 4.0 mg a.i./L treatment groups. At test termination, mortality in the 6.9, 10 and 18 mg a.i./L treatment group were 90, 100 and 100%, respectively.

Results with reference substance (positive control) : no data

Applicant's summary and conclusion

Conclusions

- The protocol states that fish will weight between 0.1 and 3.0 grams. 5 fish were slightly below the minimum due to the closed bottle design of the study.
- The protocol states that two replicate test chambers will be maintained in each treatment and control group, with 10

sheepshead minnows in each chamber. In one replicate of the 4.3 mg a.i./L treatment group, there were 9 fish. No adverse effect on the results of the study.
- Analytical sample not collected after 100% mortality occured in the 12 mg a.i./L treatment group. No adverse effect on the results of the study.

Executive summary

Acute toxicity to Cyprinodon variegatus has been investigated through EPA OPPTS 850.1075; U.S. Environmental Protection Agency Standard Evaluation Procedure for Acute Toxicity Test for Estuarine and Marine Organisms and ASTM Standard E729-96. Cyprinodon variegatus were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 2.6 - 4.3 - 7.2 - 12 and 20 mg active ingredient (a.i.)/L. 96h-LC50 was 5.6 mg/L and 96h-NOEC was < 2.3 mg/L.

4.4.2 **Short-term toxicity to aquatic invertebrates**

[Study 1] Noack 2007/K1 KS/Acute daphnia

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	Key study	1 (reliable without restrictions)	Noack M	2007	DIMETHYL DISULFIDE TC: Acute immobilization (semi-static, 48h) to Daphnia magna

Materials and methods

Test guideline

Qualifier	**Guideline**	**Deviations**	**GLP Compliance**
according to	EPA OPPTS 850.1010 (Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids)	no	yes (incl. certificate)
according to	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	no	
according to	EU Method C.2 (Acute Toxicity for Daphnia)	no	

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.89%

Analytical monitoring : yes

Details on sampling

- Preparation of standards: A stock solution of the standard of 1000 mg/L was prepared in methanol. Dilutions (9 concentrations) were prepared in methanol in the range of 0.4 to 100 mg/L. 0.1 mL of each dilution was diluted with 9.9 mL aqueous sodium chloride (10 g/L) and used for calibration.
- Preparation of samples: Aliquots of the samples were diluted in 20 mL headspace vials pre-filled with aqueous sodium chloride (10 g/L in demineralized water).

Details on analytical methods

- Separation method: GC- Detection method: Mass Selective (MS/MS)
- Chromatography on a Factor Four VF-35ms capillary column; 30 m, i.d.: 0.25 mm, film thickness: 0.25 µm
- LOQ: 10.0 µg DMDS/L

- Linearity: checked by analysis of standards and plotting a calibration graph of peak area versus concentration.
- Accuracy: 5 replicates of demineralised water containing 10 g NaCl/L fortified with 10, 100 and 100000 µg/L of the test item, respectively, and two blank samples were prepared and analysed.
- Precision: mean recovery rates are between 86 and 106 %. relative standard deviation are lower than 20 %.
- Specificity: 2 blank samples were used to prove specificity and blank values being < 30 % of the LOQ.

Vehicle : yes

Details on test solutions

The solution (100 mg/L test item) was prepared with dilution water in 2-L glass flask one day prior to application. For preparation of the stock solution 94 µL/L test item was applied to the dilution water. The stock solution was stirred for 24h at room temperature. For the preparation of the test, concentrations aliquots of the stock solution were taken from the middle of the flask.

Test organisms

Test organisms (species) : Daphnia magna

Details on test organisms

- Strain: Daphnia magna STRAUS (clone 5)
- Source: Institut für Wasser-, Boden- und Lufthygiene
- Age: 2h - 24h old
- Food type: ad libitum with a mix of Desmodesmus subscapitatus and Chlorella vulgaris
- Amount: algae cell density of > 10^6 cells/mL
- Frequency: at least 5 times per week
- Acclimation period: maintained in dilution water at the test temperature at least 48h prior to the start of the test.

Study design

Test type : semi-static

Water media type : freshwater

Limit test : no

Total exposure duration : 48 h

Test conditions

Hardness : 160 - 180 mg CaCO3/L

Test temperature : 20 ± 2ºC (mean value throughout exposure: 20.9 °C)

pH : between 7.70 and 8.04

Dissolved oxygen : between 82 and 96 %

Salinity : no data

Nominal and measured concentrations

Nominal concentrations: 0.970 - 2.13 - 4.70 - 10.3 - 22.7 and 50 mg/L (spacing factor 2.2)
Measured concentrations: 0.618 - 1.79 - 3.69 - 8.25 - 17.4 and 45.1 mg/L

Details on test conditions

TEST SYSTEM- 2-3 L glass vessels with approximately 1.8 L culture medium
- Glass flasks filled up with the test item solutions only having a small headspace to reduce losses of the test item (volatility of the test item)
- No. of organisms per vessel: 5
- No. of vessels per concentration (replicates): 4
- Renewal: after 24h

TEST MEDIUM / WATER PARAMETERS
- Conductivity: 429 - 433 µS/cm
- Intervals of water quality measurement: 24h
OTHER TEST CONDITIONS
- No feeding during test
- Photoperiod: 16/8h light/dark cycle
- Light intensity: diffuse light, illumination range max. 20 µE.m^{-2}.s^{-1}; 30 min twilight illumination

Reference substance (positive control) : yes (potassium dichromate)

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
48 h	EC50	1.82 mg/L	meas. (geom. mean)	test mat.	mobility	95% CL: 1.78 - 1.86 mg/L

Results with reference substance

EC50-24h = 1.99 mg/L (within the prescribed concentration range of 0.6 - 2.1 mg/L according to OECD 202)

Applicant's summary and conclusion

Conclusions

- In the control group no Daphnia were immobilised or trapped on the surface of the water.
- The 24h-EC50 of the reference item was in the range of 1.0 to 2.5 mg/L.
- The dissolved oxygen concentration was between 60 and 105%.
- The deviation of the pH-value from the initial value did not vary by more than one unit.
- Recovery rates of dimethyl disulfide TC were in the range of 47 to 105 % of the initial concentrations. Therefore all toxicity values are presented as mean measured concentrations. Among the replicates of each concentration the measured concentration did not vary more than + or - 20 % except test item concentration 0.970 mg/L at test start (+ or - 23.6 %).

Executive summary

Acute toxicity to Daphnia magna has been investigated through EPA OPPTS 850.1010, OECD Guideline 202 and EU Method C.2 (Acute Toxicity for Daphnia). Daphnia magna were exposed for 48 hours to an aqueous solution of dimethyl disulfide TC at different nominal concentrations of 0.970 - 2.13 - 4.70 - 10.3 - 22.7 and 50 mg/L. 48h-EC50 was 1.82 mg/L and 48h-NOEC was 0.618 mg/L.

[Study 2] Thiebaud 1996/K1/Acute daphnia

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Supporting study	1 (reliable without restrictions)	Thiebaud H	1996	DISULFURE DE DIMETHYLE: Toxicité aiguë vis-à-vis des daphnies

Materials and methods

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
according to	EU Method C.2 (Acute Toxicity for Daphnia)		yes
according to	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)		

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Analytical monitoring : yes

Details on sampling

Preparation: dilution at 50% in acetone

Details on analytical methods

- Separation method: GC
- Column: capilar; CP SIL 19 CB, 60m lenght, 0.25 µm diameter
- Detector: FID
- Internal calibration; range = 0.428 mg/L to 21.44 mg/L
- Detection limit: 0.18 mg/L
- Quantification limit: 0.54 mg/L
- Repeatability at 0.428 mg/L; CV = 8.23 %
- Accuracy: tested at 1.35 mg/L (14%)

Vehicle : yes

Details on test solutions

Preparation: a satured solution was prepared by shaking 100 mg of disulfure de dimethyle in 1 L of test medium for 24h at 20°C.

Test organisms

Test organisms (species) : Daphnia magna

Details on test organisms

- Source: growth in the laboratory- Age: < 24h

Study design

Test type : static

Water media type :freshwater

Limit test :no

Total exposure duration: 48 h

Test conditions

Hardness : no data

Test temperature : 18 - 20 °C

pH : 7.88 - 8.03

Dissolved oxygen : no data

Salinity : no data

Nominal and measured concentrations

Nominal concentrations: 3.3 - 4.0 - 4.8 - 5.8 - 6.9 - 8.3 - 10.0 - 12.0 and 14.4 mg/L

Details on test conditions

- Test vessel: 120 mL glass bottles stoppered with Teflon bungs and sealed with aluminium caps
- No. of organisms per vessel: 10
- No. of vessels per concentration: 4

Reference substance (positive control) : yes (potassium dichromate)

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
48 h	EC50	7 mg/L	meas. (not specified)	test mat.	mobility	95% confidence interval: 6.5 - 7.6 mg/L
24 h	EC50	13.4 mg/L	meas. (not specified)	test mat.	mobility	

Results with reference substance

Potassium dichromate: 24h-EC50 = 0.95 mg/L, realized the 30 November 1995

Applicant's summary and conclusion

Conclusions

- O2 concentration > 2 mg/L after 48h and pH did not vary by more than 1 unit.
- No more than 10 % of the daphnids immobilized in the control.
- Final concentrations did not remain within the 80%-120% range of initial measured concentrations.

Executive summary

Acute toxicity to Daphnia magna was investigated through OECD Guideline 202 and EU Method C.2.
Daphnia magna were exposed for 48 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 3.3 - 4.0 - 4.8 - 5.8 - 6.9 - 8.3 - 10.0 - 12.0 and 14.4 mg/L. 48h-EC50 was 7 mg/L.

[Study 3] Minderhout 2008/K1 KS/Acute saltwater mysid

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable without restrictions)	Minderhout T., Kendall T.Z., Krueger H.O	2007	DIMETHYL DISULFIDE: A 96-hour static-renewal acute toxicity test with the saltwater mysid (americamysis bahia)

Materials and methods

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
according to	EPA OPPTS 850.1035 (Mysid Acute Toxicity Test)		yes (incl. certificate)
according to	other guideline: U.S. Environmental Protection Agency, Standard Evaluation Procedure: Acute Toxicity Test for Estuarine and Marine Organisms		
according	other guideline: ASTM Standard E729-96: Standard		

to	Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates and Amphibians		

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.6%

Analytical monitoring : yes

Details on sampling

Samples were collected from the newly prepared test solutions at the beginning of the test and at approximately 48 hours at renewal, and from each test chamber of each treatment and control group (old solution) prior to renewal and at termination of the test to measure concentrations of the test substance. When 100% mortality occurred in a treatment group, samples were collected to measure the concentrations of the test substance, and then discontinued. All samples were collected at mid-depth, placed into autosampler vials, and processed immediately for analysis.

Details on analytical methods

- Separation method: HPLC with ultraviolet detection at 200 nm
- Chromatographic separations were achieved using a YMC-Pack ODS-AM column (150 mm x 4.6 mm, 3-µm particle size)
- Flow rate: 1.000 mL/min
- Wavelength: 200 nm
- Limit of quantitation (LOQ) was defined as 0.500 mg a.i./L, calculated as the product of the concentration of the lowest calibration standard (0.500 mg a.i./L) and the dilution factor of the matrix blank samples (1.00)

Vehicle: yes

Details on test solutions

Individual test solutions were prepared in the approximately 2-L class A glass volumetric flasks at nominal concentrations of 1.1, 2.3, 4.5, 9.0 and 18 mg a.i./L. The solutions were mixed on stir plates for approximately 15 minutes. After mixing, the test solutions were decanted into the approximately 500-mL French square test chambers to nearly filling. The mysids were then transferred into the test chambers and the test chambers were completely filled. At test initiation and termination, all solutions appeared clear and colorless. All test solutions were adjusted to 100% active ingredient during preparation, based on the test substance purity (99.6%) and corrected for the specific gravity of 1.063.

Test organisms

Test organisms (species) : Americamysis bahia

Details on test organisms

TEST ORGANISM
- Saltwater Mysid
- Age: < 24 hours old
- Source: Wildlife International, Ltd., Easton, Maryland
- Acclimation conditions: during the 2-week period preceding the test, water temperature in the cultures ranged from 23.9 to 26.3ºC. The pH of the water ranged from 8.0 to 8.3. Dissolved oxygen concentrations ranged from 6.5 to 7.7 mg/L (≥89% of saturation). Salinity of the water was 20 parts per thousand. During the holding period, the adults showed no sign of disease or stress.
- Feeding: brine shrimp (Artemia sp.) nauplii (Brine Shrimp Direct, Ogden, Utah), daily, occasionally enriched with ALGAMAC-2000 (Aquafauna, Hawthorn, California) to prevent cannibalism. During the test, the juvenile mysids were fed live brine shrimp (Artemia sp.) nauplii at test initiation and at renewal on day 2.

Study design

Test type: semi-static

Water media type: saltwater

Limit test : no

Total exposure duration : 96 h

Test conditions

Hardness: no data

Test temperature : 25 ± 2ºC

pH : ranged from 7.9 to 8.3

Dissolved oxygen : D.O. remained ≥ 4.5 mg/L (≥61% of saturation) throughout the test.

Salinity : 19 and 20‰ at test initiation and termination respectively

Nominal and measured concentrations

Nominal test concentrations: 1.1 - 2.3 - 4.5 - 9.0 and 18 mg active ingredients (a.i.)/LMean measured concentrations: 1.1 - 2.5 - 4.3 - 10 and 19 mg a.i./L

Details on test conditions

TEST SYSTEM
- No. of organisms per vessel: 10.
- 2 replicate test chambers.
- Total of 20 mysids per test concentration.
- Test chamber: 500-mL glass French square bottles with teflon-lined lids containing approximately 500 mL of test solution.
- Depth of the test water in a representative chamber was 18.6 cm

WATER PARAMETERS
- The water used for culturing and testing was natural seawater that was filtered and diluted to a salinity of approximately 20‰ with well water.

OTHER TEST CONDITIONS
- Fluorescent light bulbs that emit wavelengths similar to natural sunlight used for illumination of the culture and test chambers.
- A 30-minute transition period of low light intensity was provided at the beginning and end of the 16-hour light period to avoid sudden changes in lighting.
- Photoperiod: 16 hours of light and 8 hours of darkness.
- Light intensity measured at test initiation was 412 lux at the surface of the water of one representative test chamber.
- Test solutions renewed at mid-point of the test (48h).

Reference substance (positive control) : no data

Results and discussions

Effect concentrations

Duration	**Endpoint**	**Effect conc.**	**Nominal/Measured**	**Conc. based on**	**Basis for effect**	**Remarks (e.g. 95% CL)**
96 h	LC50	5 mg/L	meas. (geom. mean)	test mat.	mortality	95% confidence interval: 2.5 to 10 mg a.i./L
96 h	NOEC	2.5 mg/L	meas. (not specified)	test mat.	mortality	

Details on results

All mysids in the negative control group appeared healthy and normal throughout the test, with the exception of one erratic swimmer noted at test termination. All mysids in the 2.5 mg a.i./L treatment group also appeared normal throughout the test with no mortalities or overt signs of toxicity observed. One mysid in the 1.1 mg a.i./L was missing and presumed dead at test termination. However, it was considered an incidental death and not treatment-related. At test termination, mortality in the 4.3, 10 and 19 mg a.i./L treatment groups was 35, 100 and 100%, respectively.

Results with reference substance : no data

Applicant's summary and conclusion

Conclusions

Deviations:
- The analytical samples were collected in the 18 mg/L treatment replicates at 24h when 100% mortality occured, rather than at the next sampling interval (48h), but no impact on the results.
- During the test, mysids fed at test initiation and at renewal at 48h rather than fed at least once daily, due to the close-bottle design of the study, but no impact on the results.
- The min-max temperature readings were not recorded on day 2.

Executive summary

Acute toxicity to Saltwater Mysid (Americamysis bahia) has been investigated through EPA OPPTS 850.1035, ASTM Standard E729-96 and U.S. Environmental Protection Agency, Standard Evaluation Procedure: Acute Toxicity Test for Estuarine and Marine Organisms. Americamysis bahia were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 1.1 - 2.3 - 4.5 - 9.0 and 18 mg active ingredients (a.i.)/L. 96h-LC50 was 5 mg/L and 96h-NOEC was 2.5 mg/L.

[Study 4] Minderhout 2008/K1/Acute Oyster

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	supporting study	1 (reliable without restrictions)	Minderhout T., Kendall T.Z., Krueger H.O	2007	DIMETHYL DISULFIDE: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica)

Materials and methods

Test guideline

Qualifier	**Guideline**	**Deviations**	**GLP Compliance**
according to	EPA OPPTS 850.1025 (Bivalve Acute Toxicity (shell deposition test))		yes (incl. certificate)
according to	other guideline: U.S. Environmental Protection Agency, Standard Evaluation Procedure: Acute Toxicity Test for Estuarine and Marine Organisms (Mollusc 96-Hour Flow-Through Shell Deposition Study)		
according to	other guideline: ASTM Standard E729-96: Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates and Amphibians		

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Analytical monitoring : yes

Details on sampling

Samples were collected from each treatment and control group two days prior to test initiation to confirm the operation of the diluter after conditioning for approximately 24 hours. Two additional sets of samples were collected from each treatment and control group at approximately 0, 48 and 96 hours to measure concentrations of the test substance.

Details on analytical methods

- Separation method: HPLC with ultraviolet detection at 200 nm.
- Chromatographic separations were achieved using a YMC-Pack ODS-AM column (150 mm x 4.6 mm, 3-µm particle size).
- Flow rate: 1.000 mL/min
- Wavelength: 200 nm
- Limit of quantitation (LOQ) was defined as 2.00 mg a.i./L, calculated as the product of the concentration of the lowest calibration standard (2.00 mg a.i./L) and the dilution factor of the matrix blank samples (1.00).

Vehicle : yes

Details on test solutions

The primary stock solutions were prepared by dissolving the test substance in dimethyl formamide at nominal concentrations of 38, 75, 150, 300 and 600 mg a.i./mL. The stock solutions were injected into the diluter mixing chambers (at a rate of 35.0 µL/min) where they were mixed with filtered saltwater and feed (at a rate of 332 and 18 mL/minute, respectively) to achieve the desired test concentrations. All test solutions were adjusted to 100% active ingredient during preparation, based on the test substance purity and corrected for the specific gravity of 1.063. All of the test solutions appeared clear and colorless in the mixing chambers and test chambers at test initiation.

<u>Test organisms</u>

Test organisms (species) : other aquatic mollusc: Eastern Oyster (Crassostrea virginica)

Details on test organisms

TEST ORGANISM
- Source: Circle C Oyster Ranch Ridge, Maryland
- Mean length: 44.7 ± 1.54 mm (range: 42.8 – 47.8 mm); acceptable 30 to 50 mm size criterion.
- Received 10 days prior to test initiation and held in filtered saltwater from the same source as used during the test
- Acclimation conditions: during the ten-day holding period preceding the test, the oysters showed no sign of disease or stress. Water temperatures in the holding tank ranged from 19.5 to 21.1ºC. The pH of the water ranged from 7.6 to 8.1. Dissolved oxygen concentrations ranged from 6.7 to7.7 mg/L (≥84% of saturation). Salinity of the water was 20 parts per thousand.
- Feeding: suspension of marine microalgae (Reed Mariculture, Inc., Campbell, California), provided continuously during holding at a nominal rate of 2.9 x 109 cells/oyster/day and during the test at a nominal rate of $5.8*10^9$ cells/oyster/day.

<u>Study design</u>

Test type : flow-through

Water media type: saltwater

Limit test : no

Total exposure duration : 96 h

<u>Test conditions</u>

Hardness : no data

Test temperature : 20 ± 2ºC

pH : ranged from 8.0 to 8.1

Dissolved oxygen : remained ≥6.1 mg/L (≥76% of saturation) throughout the test.

Salinity : 20‰ at test initiation and termination

Nominal and measured concentrations

Nominal test concentrations: 3.8 - 7.5 - 15 - 30 and 60 mg active ingredients (a.i.)/L
Mean measured concentrations: 3.0 - 5.9 - 12 - 23 and 50 mg a.i./L

Details on test conditions

TEST SYSTEM
- No. of organisms per vessel: 20
- Test chamber: 54-L glass aquaria filled with approximately 27 L of test water
- Depth of the test water in a representative chamber was 13.9 cm
- Continuous-flow diluter used to deliver each concentration of the test substance, a solvent (dimethyl formamide) control, and a negative (dilution water) control.
- During the test, each chamber received approximately 19 volume additions of test water every 24 hours.

WATER PARAMETERS
- The water used for culturing and testing was natural seawater that was filtered and diluted to a salinity of approximately 20‰ with well water.

OTHER TEST CONDITIONS
- Fluorescent light bulbs that emit wavelengths similar to natural sunlight used for illumination of the culture and test chambers.
- Photoperiod: 16 hours of light and 8 hours of darkness.
- Light intensity measured at test initiation was 249 lux over one representative test chamber.

Reference substance (positive control) : no data

<u>Results and discussions</u>

Effect concentrations

Duration	**Endpoint**	**Effect conc.**	**Nominal/Measured**	**Conc. based on**	**Basis for effect**	**Remarks (e.g. 95% CL)**
96 h	EC50	14 mg/L	meas. (arithm. mean)	test mat.	mortality	95% confidence interval: 11 to 15 mg a.i./L
96 h	NOEC	3 mg/L	meas. (not specified)	test mat.	mortality	

Details on results

At test termination, all solutions appeared slightly cloudy and green due to algae feed in the mixing chambers and slightly cloudy and green due to algae feed and waste in the test chambers. There were no mortalities among oysters in the control and the 3.0 to 12 mg a.i./L treatment groups during the test. The live oysters appeared normal throughout the 96-hour exposure period, with the exception of four oysters in the 12 mg a.i./L. These four oysters were slow to close their valves when gently prodded. Percent mortality of oyster in the 23 and 50 mg a.i./L were 40 and 80%, respectively. In the 23 mg a.i./L treatment group, three of the twelve live oysters were slow to close their valves when gently prodded. The four live oysters in the 50 mg a.i./L treatment group also were slow to respond. After 96-hours, the mean shell deposition in the negative and solvent control groups was 3.2 and 2.5 mm, respectively. When the shell deposition data for the negative control was compared with the solvent control, no statistically significant differences were found at the 95% level of confidence. Therefore, the control data were pooled for comparison with the treatment groups. Mean shell deposition in the 3.0, 5.9, 12, 23 and 50 mg a.i./L treatment groups was 2.9, 2.2, 1.7, 0.2 and 0.0 mm, respectively. Percent inhibition of shell growth was calculated relative to the pooled control data. Inhibition of shell growth in the 3.0, 5.9, 12, 23 and 50 mg a.i./L treatment groups was 0.62, 24, 41, 94 and 100%, respectively.

Results with reference substance : no data

<u>Applicant's summary and conclusion</u>

Conclusions

Deviation: the continuous delivery algal suspension was interrupted by inadvertence from the afternoon of day 7 until the morning of day 8 (18.5hours) of the acclimatation period, but there has no adverse impact upon the results or interpretation of the study.

Executive summary

Acute toxicity to Eastern Oyster (Crassostrea virginica) has been investigated through EPA OPPTS 850.1025, ASTM Standard E729-96 and U.S. Environmental Protection Agency, Standard Evaluation Procedure: Acute Toxicity Test for

Estuarine and Marine Organisms (Mollusc 96-Hour Flow-Through Shell Deposition Study).
Crassostrea virginica were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 3.8 - 7.5 - 15 - 30 and 60 mg active ingredients (a.i.)/L. 96h-EC50 was 14 mg/L and 96h-NOEC was 3 mg/L.

[Study 5]: Seppovaara 1970/K4/Short-term toxicity to aquatic invertebrates

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**	**Bibliographic source**
Publication	/	4 (not assignable)	SEPPOVAARA, O. and HYNNINEN, P.	1970	On the toxicity of sulfate-mill condensates	Papper och Trä, 1, 11-23

Materials and methods

Principles of method if other than guideline
Method according to: WERNER, A.E.: Sulphur compounds in kraft pulp mill effluents.Can. Pulp paper Ind., 1963, 16, 3, 35-43.

GLP compliance: no

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Analytical monitoring : no

Test organisms

Test organisms (species) : Daphnia pulex

Study design

Test type : static

Test conditions

Test temperature : 20°C

Details on test conditions
The test was made in glass cylinder of 110 ml capacity. The volume of the test solution was 100 mL.

Results and discussions

Effect concentrations

Duration	**Endpoint**	**Effect conc.**	**Nominal/Measured**	**Conc. based on**	**Basis for effect**	**Remarks (e.g. 95% CL)**
48 h	EC50	4 mg/L	no data	test mat.	mortality	
48 h	other: EC50, 24h	15 mg/L	no data	test mat.	mortality	

[Study 6] Werner 1963/K4/Short-term toxicity to aquatic invertebrates

Study Reference

Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**	**Bibliographic source**
Publication	/	4 (not assignable)	WERNER, A.E.	1963	Sulphur compounds in kraft pulp mill effluents	Can. Pulp paper Ind., 1963, 16, 3,35-43

Materials and methods

Principles of method if other than guideline
Method: other

GLP compliance : no

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Analytical monitoring : no

Test organisms

Test organisms (species) : Daphnia pulex

Study design

Test type: static

Test conditions

Test temperature : 21°C

Details on test conditions
Groups of 3-5 daphnia were dispensed into glass sample vials, each of which containing 5.0 ml of "culture water" at 21°C. 15.0 mL of toxic solution were added. The vials were transported in the darkness of a covered, thermostatically controlled water-bath (21 ± 0.05°C). The vials were set up in triplicate. There were 6 concentrations per chemical. The concentration series was progressively adjusted so as to approach the 50% mortality. Controls were included in each experiments to give an estimate of control-mortality.

Results and discussions

Effect concentrations

Duration	**Endpoint**	**Effect conc.**	**Nominal/Measured**	**Conc. based on**	**Basis for effect**	**Remarks (e.g. 95% CL)**
4 h	EC50	21.4 mg/L	no data	test mat.	mortality	

[Study 7] S.N.E.A.P. 1984/K4/Acute daphnia

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title	Bibliographic source
other company data	/	4 (not assignable)	Dubreuil J.P.	1984	PROCES-VERBAL D'ESSAIS-N° 84/4.381.	

Materials and methods

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
according to	ISO 6341 15 (Water quality - Determination of the Inhibition of the Mobility of Daphnia magna Straus (Cladocera, Crustacea))	no data	No

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Analytical monitoring: no

Details on sampling : no data

Details on analytical methods : no data

Details on test solutions : no data

Test organisms

Test organisms (species) : Daphnia sp.

Details on test organisms : no data

Study design

Test type : no data

Water media type: no data

Total exposure duration: 24 h

Test conditions

Hardness : no data

Test temperature : no data

pH : no data

Dissolved oxygen : no data

Salinity : no data

Nominal and measured concentrations : no data

Details on test conditions : no data

Reference substance (positive control) : yes (potassium bichromate)

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
24 h	EC50	5 mg/L	no data	test mat.	mortality	

Details on results

This substance can be considered as toxic, as 24h-EC50 is comprised between 1 and 10 mg/L.

Results with reference substance

Control performed on potassium bichromate (24h-EC50 = 1.1 mg/l)

Applicant's summary and conclusion

Executive summary

Acute toxicity to Daphnia sp. has been investigated through ISO 6341 15. Daphnia sp. were exposed for 24 hours to a solution of dimethyl disulfide. 24h-EC50 was 5 mg/L.

4.4.3 Algal growth inhibition tests

[Study 1] Anabaena_Minderhout 2008/K1 KS/Toxicity to algae

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable without restrictions)	Minderhout T., Kendall T.Z., Krueger H.O	2008	DIMETHYL DISULFIDE: A 96-HOUR TOXICITY TEST WITH THE FRESHWATER ALGA (Anabaena flos-aquae)

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 201 (Alga, Growth Inhibition Test)	
according to	EU Method C.3 (Algal Inhibition test)	
according to	EPA OPPTS 850.5400 (Algal Toxicity, Tiers I and II)	

GLP compliance : yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.6%

Analytical monitoring : yes

Details on sampling

Prior to test initiation, the concentration of algal cells in the stock culture was $1.06*10^6$ cells/mL. Therefore, an inoculum volume of 1.5 mL of the stock culture was added to each test chamber to achieve a nominal concentration of approximately 5000 cells/mL at test initiation.

Details on analytical methods

- Separation method: HPLC with ultraviolet detection at 200 nm
- Chromatographic separations were achieved using a YMC-Pack ODS-AM column (150 mm x 4.6 mm, 3-µm particle size)
- Flow rate: 1.000 mL/min
- Wavelength: 200 nm
- Limit of quantitation (LOQ) was defined as 0.100 mg a.i./L, calculated as the product of the concentration of the lowest calibration standard (0.100 mg a.i./L) and the dilution factor of the matrix blank samples (1.00)

Vehicle : yes

Details on test solutions

Individual test solutions were prepared in the 4-L glass aspirator bottles at nominal concentrations of 0.15, 0.38, 0.96, 2.4, 6.0 and 15 mg a.i./L. The calculated amount of test substance was measured using syringes and delivered into the bottles containing freshwater algal medium. The bottles were sealed with parafilm. The solutions were mixed using magnetic stir bars and stir plates for approximately 15 minutes. After mixing, test solutions were transferred into the test chamber, a 300-mL glass bottle with a glass stopper with two glass marbles. The test chambers were filled completely to limit headspace. All test solutions were adjusted to 100% active ingredient during preparation, based on the test substance purity (99.6%) and the specific gravity of 1.063.

Test organisms

Test organisms (species) : Anabaena flos-aquae

Details on test organisms

- Freshwater alga
- Source: University of Toronto
- Growth in culture medium for at least two weeks prior to test initiation
- The culture was last transferred to fresh medium four days prior to test initiation

Study design

Test type : static

Water media type : freshwater

Limit test : no

Total exposure duration : 96
h

Test conditions

Hardness : no data

Test temperature : 24 ± 2°C

pH : The pH of all the test solutions ranged from 7.6 to 7.8 at test initiation and from 8.9 to 10.0 at test termination.

Dissolved oxygen : no data

Salinity : no data

Nominal and measured concentrations

Nominal test concentrations: 0.15 - 0.38 - 0.96 - 2.4 - 6.0 and 15 mg active ingredients (a.i.)/L
Mean measured concentrations: 0.17 - 0.34 - 0.80 - 1.9 - 5.5 and 14 mg a.i./L

Details on test conditions

- 24 hours per day of cool-white fluorescent lighting at an intensity of 2,150 ± 10% lux
- Test chambers were 300-mL sterile glass BOD bottles with glass stoppers. They were completely filled with test

solutions or control medium.
- Each test bottle also contained two glass marbles as an aid to mixing.

Reference substance (positive control) : no data

Any other information on materials and methods incl. tables

The test bottles were shaken continuously at approximately 100 rpm with the exception of approximately 24 hours between Days 1 and 2 of the test when one of the shaker tables was inadvertently not turn back on after the samples were collected on Day 1 of the test. A visual examining the cell counts data indicates no significant difference in variability among the affected and non-affected replicates.

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	NOEC	0.17 mg/L	meas. (arithm. mean)	act. ingr.	growth rate	
96 h	EC50	6.7 mg/L	meas. (geom. mean)	act. ingr.	growth rate	

Details on results

After 96 hours of exposure, there were no signs of adherence of cells to the test chambers in the negative control or in any treatment group. There were noticeable flocculation/aggregations in the negative control, 0.17, 0.34, 0.80 and 1.9 mg a.i./L treatment groups. However, no aggregations were noted in the 5.5 and 14 mg a.i./L treatment groups. Cell morphology was examined during haemacytometer cell counts. Enlarged cells were observed in the 1.9, 5.5 and 14 mg a.i./L samples collected on Days 2, 3 and 4 of the test.

Results with reference substance (positive control) : no data

Applicant's summary and conclusion

Conclusions

The mean cell density in the control flasks increased by a factor of greater than 16 within three days. The test was therefore considered valid.

Executive summary

Acute toxicity of DMDS to Anabaena flos-aquae has been investigated through OECD Guideline 201, EU Method C.3 and EPA OPPTS 850.5400. Anabaena flos-aquae were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 0.15 - 0.38 - 0.96 - 2.4 - 6.0 and 15 mg active ingredients (a.i.)/L. 96h-ECr50, based on growth rate, was 6.7 mg/L. 96h-NOEC, based on growth rate, was 0.17 mg/L. Validity criteria were fulfilled.

[Study 2] Navicula_Minderhout 2008/K1/Toxicity to algae

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Supporting study	1 (reliable without restrictions)	Minderhout T., Kendall T.Z., Krueger H.O	2008	DIMETHYL DISULFIDE: A 96-HOUR TOXICITY TEST WITH THE FRESHWATER DIATOM (Navicula pelliculosa)

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 201 (Alga, Growth Inhibition Test)	
according to	EU Method C.3 (Algal Inhibition test)	
according to	EPA OPPTS 850.5400 (Algal Toxicity, Tiers I and II)	

GLP compliance : yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.6%

Analytical monitoring : yes

Details on sampling

Prior to test initiation, an inoculum of the algal cells was prepared in freshwater algal medium at a concentration of approximately $1.0*10^6$ cells/mL. The concentration of algal cells was verified and 1.5 mL of the inoculum was added to each test chamber to achieve a nominal concentration of approximately 5000 cells/mL at test initiation.

Details on analytical methods

- Separation method: HPLC with ultraviolet detection at 200 nm
- Chromatographic separations were achieved using a YMC-Pack ODS-AM column (150 mm x 4.6 mm, 3-µm particle size)
- Flow rate: 1.000 mL/min
- Wavelength: 200 nm
- Limit of quantitation (LOQ) was defined as 1.00 mg a.i./L, calculated as the product of the concentration of the lowest calibration standard (1.00 mg a.i./L) and the dilution factor of the matrix blank samples (1.00)

Vehicle : yes

Details on test solutions

Individual test solutions were prepared in 6-L glass flasks at nominal concentrations of 2.3, 3.9, 6.5, 11, 18 and 30 mg a.i./L. The calculated amount of test substance was measured using syringes and delivered into the flasks containing measured volume of freshwater algal medium. The flasks were sealed with parafilm. The solutions were mixed using magnetic stir bars and stir plates for approximately 40 minutes. After mixing, each test chamber was filled completely with test solution to minimized headspace. All test solutions were adjusted to 100% active ingredient during preparation, based on the test substance purity (99.6%) and corrected for the specific gravity of 1.063.

Test organisms

Test organisms (species) : Navicula pelliculosa

Details on test organisms

- Freshwater diatom
- Source: Wildlife International, Ltd. Cultures, Easton, Maryland
- Growth in culture medium for at least two weeks prior to test initiation
- The culture was transferred to fresh medium four days prior to test initiation

Study design

Test type : static

Water media type : freshwater

Limit test : no

Total exposure duration : 96 h

Test conditions

Hardness : no data

Test temperature : 24 ± 2°C

pH : The pH ranged from 7.4 to 7.5 at test initiation and from 8.2 to 10.7 at test termination

Dissolved oxygen : no data

Salinity : no data

Nominal and measured concentrations

Nominal test concentrations: 2.3 - 3.9 - 6.5 - 11 - 18 and 30 mg active ingredients (a.i.)/L
Mean measured concentrations: 2.0 - 3.9 - 5.5 - 9.5 - 15 and 27 mg a.i./L

Details on test conditions

- Four replicate test chambers were maintained in each treatment and control group.
- Test chambers were 300-mL sterile glass BOD bottles with glass stoppers, and were completely filled with test solutions or control medium.
- Each test bottle also contained two glass marbles as an aid to mixing.
- Test bottles shaken continuously at approximately 100 rpm.
- 24 hours per day of cool-white fluorescent lighting at an intensity of 4300 ± 10% lux

Reference substance (positive control) : no data

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
96 h	EC50	25 mg/L	meas. (arithm. mean)	act. ingr.	growth rate	
96 h	NOEC	15 mg/L	meas. (arithm. mean)	act. ingr.	growth rate	

Details on results

After 96 hours of exposure, there were noticeable adherences of cells to the test chambers in the negative control and 2.0 to 15 mg a.i./L treatment groups. There were no signs of adherence of cells to the test chambers in the 27 mg a.i./L treatment group. The aggregation/flocculation of algae were noted in the negative control and the 2.0 and 3.9 mg a.i./L treatment groups, but were not observed in the 5.5 to 27 mg a.i./L treatment groups. There were no noticeable changes in cell morphology at all test concentrations when compared to the control.

Results with reference substance (positive control) : no data

Applicant's summary and conclusion

Conclusions

The mean cell density in the control bottles increased by a factor of at least 16 within three days.

Executive summary

Acute toxicity to Navicula pelliculosa has been investigated through OECD Guideline 201, EU Method C.3 and EPA OPPTS 850.5400. Navicula pelliculosa were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 2.3 - 3.9 - 6.5 - 11 - 18 and 30 mg active ingredients (a.i.)/L. 96h-ErC50 and 96h-NOEC, based on growth rate, were 25 mg/L and 15 mg/L respectively.

[Study 3] Pseudokirchneriella_Scheerbaum 2007/K1/Toxicity to algae

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	supporting study	1 (reliable without restrictions)	Scheerbaum D.	2007	DIMETHYL DISULFIDE TC: Alga, growth inhibition test with Pseudokirchneriella subcapitata, 72h

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 201 (Alga, Growth Inhibition Test)	no
according to	EU Method C.3 (Algal Inhibition test)	no
according to	EPA OPPTS 850.5400 (Algal Toxicity, Tiers I and II)	no

GLP compliance : yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.89%

Analytical monitoring : yes

Details on sampling

- Preparation of standards: A stock solution of the standard of 1000 mg/L was prepared in methanol. Dilutions (9 concentrations) were prepared in methanol in the range of 0.4 to 100 mg/L. 0.1 mL of each dilution was diluted with 9.9 mL aqueous sodium chloride (10 g/L) and used for calibration.
- Preparation of samples: Aliquots of the samples were diluted in 20 mL headspace vials pre-filled with aqueous sodium chloride (10 g/L in HPLC water).

Details on analytical methods

- Separation method: GC
- Detection method: Mass Selective (MS/MS)
- Chromatography on a Factor Four VF-35ms capillary column; 30 m, i.d.: 0.25 mm, film thickness: 0.25 µm- External calibration
- LOQ: 10.0 µg DMDS/L
- Linearity: checked by analysis of standards and plotting a calibration graph of peak area versus concentration.
- Accuracy: 5 replicates of demineralised water containing 10 g NaCl/L fortified with 10, 100 and 100000 µg/L of the test item, respectively, and two blank samples were prepared and analysed.
- Precision: mean recovery rates are between 95 and 106 %. Relative standard deviation were lower than 20 %.
- Specificity: 2 blank samples were used to prove specificity and blank values being < 30 % of the LOQ.

Vehicle:

yes

Details on test solutions

A saturated solution was prepared in dilution water. 100 mg/L were stirred for 24h. For the preparation of the test, concentrations aliquots of the stock solution were taken from the center of the flask. 5 dilution levels were tested with a dilution factor of 1:16 - 1:8 - 1:4 - 1:2 and the saturated solution (1:1).

Test organisms

Test organisms (species) : Pseudokirchnerella subcapitata

Details on test organisms

- Unicellular freshwater green algae
- Strain: Pseudokirchnerella subcapitata HINDAK, SAG 61.81 (formerly known as Selenastrum capricornutum)
- Source: Sammlung von Algenkulturen (SAG), Göttingen
- Method of cultivation: fresh stocks were prepared every months on Z-Agar
-. Light intensity amounted 35-70 $\mu E.m^{-2}.s^{-1}$ for 24 h per day

Study design

Test type : static

Water media type : freshwater

Limit test : no

Total exposure duration : 72 h

Test conditions

Hardness : no data

Test temperature : 24 ± 2ºC (mean value throughout exposure: 23.5 °C)

pH : from 7.77 to 9.11

Dissolved oxygen : no data

Salinity : no data

Nominal and measured concentrations

Measured concentrations: 5.76 - 9.40 - 20.6 - 41.9 and 82.8 mg/L

Details on test conditions

TEST SYSTEM
- Sterile headspace flasks, volume of 119 mL, with aluminium tops with PTFE seals.
- The study was carried out in closed bottles without gas headspace due to the volatility of the test item.
- 3 replicates per concentration level, 6 per control for each day of measurement.
- 6 control replicates were used to receive a more powerful response for statistical detection.

EFFECT PARAMETERS MEASURED
- Determination of cell concentrations: Chlorophyll-a-fluorescence. No self fluorescence has been found up to 82.8 mg/L (saturated solution).

OTHER TEST CONDITIONS
- A 3 days old preculture was used as inoculum.
- Initial cell concentration of 0.5 - 1.0 * 10^4 cells/mL in the replicates.
- Light intensity: 80 - 90 $\mu E.m^{-2}.s^{-1}$
- Light regime: 24h/day light

Reference substance (positive control) : yes (potassium dichromate)

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
72 h	EC50	25.6 mg/L	meas. (arithm. mean)	test mat.	growth rate	95% CL: 23.6 - 27.8 mg/L
72 h	NOEC	9.4 mg/L	meas. (arithm. mean)	test mat.	growth rate	

Details on results

No morphological abnormalities at start of the incubation. At the end, no abnormalities, except at 41.9 - 82.8 mg/L concentrations for which cells were enlarged. After 72 h of exposure, 0.2 - 1 mL aliquots of the test medium (i.e. samples of exposed algal cells) from the dilution levels 20.6 - 82.8 mg/L and control were transferred to 10 mL untreated test medium. Algae were then allowed to grow for further 6 - 12 days under test conditions. The test item effect was observed to be reversible at these concentrations. therefore, there is potential for recovery following exposure up to 82.8 mg/L.

Results with reference substance (positive control) : no data

Applicant's summary and conclusion

Conclusions

- The cell growth increased 79-fold after 72 h in the control (required: at least 16-fold)
- The coefficient of inter-replicate variation of growth rate in the control was below 7% (CV= 5.1%)
- The coefficient of intra-replicate (section by section) variation of growth rate in the control was below 35% (CV = 25.7%)
- The water temperature during the test was in the range of 24 ± 2ºC.
- The difference from the mean was within ± 2ºC: min.: 22.9 °C, max.: 24.1 °C, mean value: 23.5 °C.
- The pH-value in the control replicates increased not higher than 1.5 units: from 7.77 to 9.11.

Executive summary

Acute toxicity to Pseudokirchnerella subcapitata was investigated through EPA OPPTS 850.5400, OECD Guideline 201 and EU Method C.3. Pseudokirchnerella subcapitata were exposed for 72 hours to an aqueous solution of dimethyl disulfide at different measured concentrations of 5.76 - 9.40 - 20.6 - 41.9 and 82.8 mg/L. For growth rate, 72h-ErC50 and 72h-NOEC were 25.6 mg/L and 9.4 mg/L respectively. All validity criteria were fulfilled.

[Study 4] Pseudokirchneriella_Thiébaud 2000/K1/Toxicity to algae

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	supporting study	1 (reliable without restrictions)	Thiebaud H, Lespagnol CC	2000	Disulfure de dimethyle: Inhibition de la croissance des algues

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	EU Method C.3 (Algal Inhibition test)	no
according to	OECD Guideline 201 (Alga, Growth Inhibition Test)	no

GLP compliance : yes

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.65%

Analytical monitoring : yes

Details on sampling

- Sterile dilution of stock solution (5 mL stock solution diluted in 500 mL of water)

Details on analytical methods

- Separation method: GC
- Detection: FID
- Column: capilar
- Detection limit: 7.6 mg/L
- Quantification limit: 25.3 mg/L
- Linearity range: 30.8 - 102.8 mg/L
- Cytofluorimeter
- Repeatability = 42.9 mg/L; CV = 2.97 %
- Accuracy = 3.15 %

Vehicle : yes

Test organisms

Test organisms (species) : Pseudokirchnerella subcapitata

Details on test organisms : Source: Culture Center of Algae and Protozoa

Study design

Test type : static

Water media type : freshwater

Limit test : no

Total exposure duration : 72h

Test conditions

Hardness : no data

Test temperature : 23 - 25 °C

pH : 7 - 7.67

Dissolved oxygen : 7.4 - 11.1 mg/L

Salinity : no data

Nominal and measured concentrations

Nominal concentrations: 5.29 - 9.53 - 17.15 - 30.86 - 55.56 - 100 mg/L

Details on test conditions

Test vessel were 120 mL glass bottles stoppered with PTFE bungs and sealed with aluminium caps. They contained 50 mL of test solution inoculated with an algal suspension so that the initial cell concentration was equal to 10^4 cells/mL. The bottles were continuously shaken under continuous light.

Reference substance (positive control) : yes (potassium dichromate)

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)

72 h	EC50	35 mg/L	nominal	test mat.	growth rate	
72 h	EC10	9.3 mg/L	nominal	test mat.	growth rate	
72 h	NOEC	10.43 mg/L	nominal	test mat.	growth rate	

Details on results

- No precipitations observed at the end of the test
- Solutions clear and homogen over the period of the test
- Normal aspect of algae at the end of the test: crescent shaped unicellular cells with an average length of 5-10 µm

Applicant's summary and conclusion

Conclusions

- The increase of cell density (R), measured in the control solution between the end and the beginning of the test, was greater than a factor of 16 (R = 328)
- Final concentrations of dimethyldisulfide were maintained within the designated limit of 80-120 % of the initial concentrations in non-inoculated flasks

Executive summary

Growth inhibition of Pseudokirchneriella subcapitata has been investigated according to EU Method C.3 (Algal Inhibition test) and OECD Guideline 201. Pseudokirchneriella subcapitata were exposed for 72 hours to an aqueous solution of dimethyl disulfure at concentrations of 5.29 - 9.53 - 17.15 - 30.86 - 55.56 - 100 mg/L. 72h-EC50 was 35 mg/L for growth rate. NOEC and EC10 were 10.43 mg/L and 9.3 mg/L for growth rate respectively.

[Study 5] Skeletonema_Minderhout 2008/K1 KS/Toxicity to algae

Study Reference Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	Key study	1 (reliable without restrictions)	Minderhout T., Kendall T.Z., Krueger H.O	2008	DIMETHYL DISULFIDE: A 96-hour toxicity test with the marine diatom (Skeletonema costatum)

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 201 (Alga, Growth Inhibition Test)	
according to	EU Method C.3 (Algal Inhibition test)	
according to	EPA OPPTS 850.5400 (Algal Toxicity, Tiers I and II)	

GLP compliance: yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.6%

Analytical monitoring:

yes

Details on sampling

Prior to test initiation, the concentration of algal cells in the stock culture was verified and was approximately 1.0 x 10^6 cells/mL. Therefore, an inoculum volume of 1.5 mL of the stock culture was added to each test chamber to achieve a nominal concentration of approximately 5000 cells/mL at test initiation.

Details on analytical methods

- Separation method: HPLC with ultraviolet detection at 200 nm
- Chromatographic separations were achieved using a YMC-Pack ODS-AM column (150 mm x 4.6 mm, 3-µm particle size)
- Flow rate: 1.000 mL/min
- Wavelength: 200 nm
- Limit of quantitation (LOQ) was defined as 0.300 mg a.i./L, calculated as the product of the concentration of the lowest calibration standard (0.300 mg a.i./L) and the dilution factor of the matrix blank samples (1.00)

Vehicle : yes

Details on test solutions

Individual test solutions were prepared in t4-L glass Pyrex® aspirator bottle at nominal concentrations of 0.58, 1.3, 2.8, 6.2, 14 and 30 mg a.i./L. The bottles were sealed with parafilm. The solutions were mixed using magnetic stir bars and stir plates for approximately 15 minutes. All test concentrations were expressed in active ingredient, based on the test substance purity (99.6%) and the specific gravity of 1.063. At test termination, no surface slicks or particules were observed in the test solutions.

Test organisms

Test organisms (species) : Skeletonema costatum

Details on test organisms

- Marine diatom
- Source: Wildlife International, Ltd. Cultures, Easton, Maryland
- Growth in culture medium for at least two weeks prior to test initiation
- The culture was transferred to fresh medium four days prior to test initiation

Study design

Test type : static

Water media type : saltwater

Limit test : no

Total exposure duration : 96
h

Test conditions

Hardness : no data

Test temperature : 20 ± 2°C

pH : The pH ranged from 7.5 to 8.0 at test initiation and from 8.2 to 10.7 at test termination.

Dissolved oxygen : no data

Salinity : no data

Nominal and measured concentrations

Nominal test concentrations: 0.58 - 1.3 - 2.8 - 6.2 - 14 and 30 mg a.i./L
Mean measured concentrations: 0.48 - 0.95 - 2.6 - 5.7 - 12 and 27 mg a.i./L

Details on test conditions

- 12 replicate test chambers were maintained in each treatment and control group.
- Test chambers were 300-mL sterile glass BOD bottles with glass stoppers, and were completely filled with test solutions or control medium.
- Each test bottle also contained two glass marbles as an aid to mixing.
- Test bottles were shaken continuously at approximately 100 rpm.
- Light: 16 hours per day of cool-white fluorescent lighting at an intensity of 4300 ± 10% lux

Reference substance (positive control) : no data

Results and discussions

Effect concentrations

Duration	**Endpoint**	**Effect conc.**	**Nominal/Measured**	**Conc. based on**	**Basis for effect**	**Remarks (e.g. 95% CL)**
96 h	other: ErC50	3.9 mg/L	meas. (arithm. mean)	act. ingr.	growth rate	
96 h	other: NOEC	0.95 mg/L	meas. (arithm. mean)	act. ingr.	growth rate	

Details on results

After 96 hours exposure to dimethyl disulfide, biomass was the most sensitive endpoint, as defined by the lowest EC50 value. The 96-hour EbC50, based on biomass, was 1.2 mg a.i./L, while the 96-hour ErC50 value was 3.9 mg a.i./L. The 96-hour NOEC, based on growth rate was 0.95 mg a.i./L.

Results with reference substance (positive control) : no data

Applicant's summary and conclusion

Conclusions

The mean cell density in the control bottles increased by a factor of at least 16 within three days.

Executive summary

Acute toxicity of DMDS to Skeletonema costatum has been investigated through OECD Guideline 201, EU Method C.3 and EPA OPPTS 850.5400. Skeletonema costatum cells were exposed for 96 hours to an aqueous solution of dimethyl disulfide at different nominal concentrations of 0.58 - 1.3 - 2.8 - 6.2 - 14 and 30 mg active ingredients (a.i.)/L. After 96 hours exposure to dimethyl disulfide, biomass was the most sensitive endpoint, as defined by the lowest EC50 value. The 96-hour EbC50, based on biomass, was 1.2 mg a.i./L, while the 96-hour ErC50 value was 3.9 mg a.i./L. The 96-hour NOEC, based on growth rate was 0.95 mg a.i./L.

4.4.4 Toxicity to aquatic plants other than algae

[Study 1] Minderhout 2008/K1 KS/Toxicity to duck weed

Study Reference Reference type	**Purpose Flag**	**Reliability**	**Author**	**Year**	**Title**
study report	Key study	1 (reliable without restrictions)	Minderhout T., Kendall T.Z., Krueger H.O	2008	Dimethyl disulfide : a 7-day static-renewal toxicity test with a duck weed (Lemna gibba G3)

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 221 (Lemna sp. Growth Inhibition test)	
according to	EPA OPPTS 850.4400 (Aquatic Plant Toxicity Test using Lemna spp. Tiers I & II))	

GLP compliance: yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier

Analytical monitoring:
yes

Details on analytical methods
- Separation method: Agilent Series 1100/1200 HPLC with ultraviolet detection
- Chromatographic separations were achieved using a YMC-Pack ODS-AM column (150 mm x 4.6 mm, 3-µm particle size)
- Flow rate: 1.000 mL/min
- Wavelength: 200 nm
- DMDS retention time: approximately 10.4 min
- Limit of quantitation (LOQ) was defined as 2.00 mg a.i./L, calculated as the product of the concentration of the lowest calibration standard (2.00 mg a.i./L) and the dilution factor of the matrix blank samples (1.00)

Vehicle : yes

Details on test solutions
Individual test solutions were prepared in the 1-L class A glass volumetric flasks at nominal concentrations of 3.1 – 6.3 – 13 – 25 – 50 and 100 mg a.i./L. The calculated amount of test substance was measured using syringes and delivered into the volumetric flasks containing 20XAAP freshwater algal medium. The flasks were sealed with parafilm. The solutions were mixed using magnetic stir bars and stir plates for approximately 15 minutes. After mixing, 200mL of test solutions were transferred into the test chamber, a 300-mL glass bottle with a screw cap with Teflon lined septum.
The appearance of the test solution, at preparation, was clear and colorless in the 3.1 to 6.3 mg a.i./L, and clear and colorless with bubbles of test substance present throughout the vortex increasing in intensity as concentration of test substance increase in the 13 to 100 mg a.i./L. All test solutions were adjusted to 100% active ingredient during preparation, based on the test substance purity (99.6%) and the specific gravity of 1.063.

Test organisms

Test organisms (species) : Lemna gibba

Details on test organisms
TEST ORGANISM
- Common name: Duckweed (Lemna gibba G3)
- Source: United States Department of Agriculture
- Maintained in culture medium at Wildlife International, Ltd., Easton, Maryland
- Growth: Lemna sp. culture medium (20X AAP) for at least 2 weeks prior to test initiation.

Study design

Test type : semi-static

Water media type : freshwater

Limit test : no

Total exposure duration: 7.0 d

Test conditions

Hardness : no data

Test temperature : 25 ± 2°C

pH : The pH ranged from 7.6 to 8.0 at test initiation and from 8.2 to 9.0 at test termination.

Dissolved oxygen : no data

Salinity : no data

Nominal and measured concentrations

Nominal concentrations: 3.1 – 6.3 – 13 – 25 – 50 and 100 mg a.i./L
Mean measured concentrations: 3.2 – 5.5 – 12 – 24 – 48 and 95 mg a.i./L

Details on test conditions

- Test chambers were sterile, 300-mL glass bottles with a screw cap with Teflon lined septum
- Contained 200mL of test solution or control medium
- 3 replicates test chambers in each treatment and control group
- 4 plants with a total of 12 fronds added to each replicate test chamber
- Continuous warm-white fluorescent lighting at an intensity of 5000 ± 750% lux
- Renewal at day 3 and 5

Reference substance (positive control) : not specified

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
7 d	EC50	31.0 mg/L	meas. (geom. mean)	act. ingr.	Frond number	
7 d	NOAEC	5.5 mg/L	meas. (geom. mean)	act. ingr.	Frond number	
7 d	EC50	46.0 mg/L	meas. (geom. mean)	act. ingr.	Biomass	

Details on results

- The toxicity of DMDS was determined by evaluating the production of plants and fronds and their general health over 7 days.
- The percentage of dead, chlorotic and necrotic fronds on days 3, 5 and 7 have been examinated.
- pH increased over the duration of the test due to photosynthetic activity of aquatic plants.

Results with reference substance (positive control) : no data

Applicant's summary and conclusion

Conclusions

Toxicity of DMDS to duckweed was low. Key values are 7d-EC50 = 31 mg a.i./L and 7d-NOEC = 5.5 mg a.i./L.

Executive summary

Acute toxicity of DMDS to Duckweed (Lemna gibba G3) has been investigated through OECD Guideline 221 and EPA OPPTS 850.4400. Duckweed were exposed for 7 days to a solution of dimethyl disulfide at different nominal concentrations of 3.1 – 6.3 – 13 – 25 – 50 and 100 mg a.i./L. 7d-EC50 was 31 mg a.i./L for frond number. 7d-NOAEC was 5.5 mg a.i./L for frond number. 7d-EC 50 was of 46 mg a.i./L for biomass. Validity criteria were fulfilled.

4.5 Chronic toxicity

4.5.1 Fish early-life stage (FELS) toxicity test

[Study 1] FELS fathead minnow, Rebstock 2011/K1/Long-term toxicity to fish.001

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable without restrictions)	Rebstock, M.	2011	Dimethyl disulfide (DMDS): Early Life-Stage Toxicity Test with the Fathead Minnow, Pimephales promelas, Under Flow-Through Conditions

Materials and methods

Life stage / endpoint studied : early-life stage: reproduction, (sub)lethal effects

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
according to	OECD Guideline 210 (Fish, Early-Life Stage Toxicity Test)		yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.7%

Analytical monitoring : yes

Details on analytical methods
Concentrations of DMDS were analytically verified using a validated HPLC/UV method. The concentrations of DMDS were measured in test solution samples (10 mL) collected two days prior to initiation (day -2) and on days 0, 7, 14, 21, 28, and 33 (termination) of the definitive test. A single replicate sample was collected from the control, vehicle control, and test substance treatments; alternating replicates were sampled at each sampling event. The concentrations of DMDS in the diluter stock solutions were determined in samples collected on the same days as the test solutions.

Vehicle : yes

Details on test solutions
PREPARATION AND APPLICATION OF TEST SOLUTION
- Method:flow through
- Eluate: The Hamilton Model 420 syringe dispenser introduced 0.4-mL volumes of the diluter stock solution to the diluter system, where the diluter stock solution volume was diluted with approximately 3,990 mL of dilution water.
- Nominal concentrations: 0 (control), 0 (vehicle control), 0.13, 0.25, 0.50, 1.0, 2.0, and 4.0 mg a.i./L
- Controls: The concentration of DMF in test solutions was 0.10 mL DMF/L. The control consisted of dilution water only.
- Chemical name of vehicle (organic solvent, emulsifier or dispersant): DMF, dimethylformamide
- Concentration of vehicle in test medium (stock solution and final test solution(s) including control(s)): stock solution: 39.9 g a.i./L every three to four days during the diluter equilibration and the definitive testing by diluting approximately

10.005 g of DMDS (9.975 g corrected for purity) to a volume of 0.25 L with dimethyl formamide (DMF).

Test organisms

Test organisms (species) : Pimephales promelas

Details on test organisms

- Species: Pimephales promelas
- Source: In-house culture
- Age/Size: embryos were <24 hours post fertilisation
- Holding: 25 ± 2ºC, light intensity 646.3 lux, under flow-through conditions, 7 volume additions to each test chamber over a 24 hour period were achieved, dissolved oxygen concentration more than 80% of the air saturation value.
- Food/Feeding: fry were initially fed live brine shrimp (Artemia sp.). A standard commercial fish food was added to the diet on study day 27, fish were fed live brine shrimp ad libitum once on study days 2 and 32 and 3 times daily during the remainder of the test. Fish were not fed during the day preceding termination.

Study design

Test type : flow-through

Water media type : freshwater

Limit test : no

Total exposure duration : 33 d

Test conditions

Hardness : 142-154 mg $CaCO_3$ /L

Test temperature : 24.4 to 25.3°C

pH : 8.0 to 8.4

Dissolved oxygen : 6.7 to 9.6 mg/L

Salinity : not applicable

Nominal and measured concentrations

Control, vehicle control (0.1 mL dimethyl formamide (DMF)/L), 0.13, 0.25, 0.50, 1.0, 2.0 and 4.0 mg a.s./L. Concentrations were selected on the basis of a preliminary study in which 0.13, 0.24, 0.50, 1.0 and 2.0 mg a.s./L (nominal) were tested.
Measured (Geometric Mean):0.0566, 0.122, 0.240, 0.549, 0.936 and 1.87 mg/L (recoveries of 44 to 55% of nominal)

Details on test conditions

Initiation of the study was to embryos <24 hours post fertilization. A sufficient number of embryos was obtained to give a group size of at least 15 embryos per replicate. There were 4 replicates for each treatment group. Day 0 post-hatch was based on ≥95% hatch in the control group. All live fry were counted and released into their respective replicate growth chambers on day 7 post-hatch and exposure continued for 28 days post-hatch. Survival was monitored daily by visually inspecting each test chamber, and any behavioural or physical changes were recorded, including abnormalities. After 28 days exposure the fish were euthanised and total length and blotted weight were measured.

Reference substance (positive control) : no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)

33 d	NOEC	0.936 mg/L	meas. (geom. mean)	test mat.	other: fry survival	
33 d	LOEC	1.87 mg/L	meas. (geom. mean)	test mat.	other: fry survival	

Details on results

Loss of the test item resulting from volatility was observed, the reported effect levels are therefore based on mean measured concentrations.

Egg hatch began on day 2 and was completed on day 5. Complete hatch (≥95% hatch in the control) was determined to be day 5 (day 0 post-hatch). There was no statistically significant reduction in hatch success observed in any of the substance treatments, as compared to the control. Based on hatching success, the NOEC was 1.87 mg a.s./L and the LOEC was >1.87 mg a.s./L.

Post-hatch survival was calculated as the percent of hatched fry that were alive at test termination (day 33; 28 days post-hatch). There was a statistically significant reduction in post-hatch survival observed at the 1.87 mg a.s./L treatment level, as compared to the control. The NOEC and LOEC values for post-hatch survival were 0.936 mg a.s./L and 1.87 mg a.s./L, respectively.

Growth of surviving fry was assessed at test termination (day 33; 28 days post-hatch) through total length and blotted wet weight measurements. Replicate values were pooled into a treatment mean using the number of individuals present at the end of the test in weighting the means. The 1.87 mg a.s./L test substance treatment was not compared to the controls for standard length and blotted wet weight due to statistically significant effects on post-hatch survival. Nonetheless there was no effect on total length or blotted weight attributable to treatment with DMDS identified. NOEC and LOEC values for total length were 1.87 mg a.s./L and >1.87 mg a.s./L, respectively whilst the NOEC and LOEC values for blotted wet weight were also 1.87 mg a.s./L and >1.87 mg a.s./L, respectively.

Hatching success in the control and vehicle control were 98 and 100%, respectively, and ranged from 95 to 100% in the DMDS treatments. Post-hatch survival of fry in the control and vehicle control was 94 and 91%, respectively, and ranged from 75 to 94% in the DMDS treatments. Mean total length was 23.3 and 23.0 mm in the control and vehicle control, respectively, and ranged from 21.6 to 22.9 mm in the DMDS treatments. Mean blotted wet weight was 0.101 and 0.107 g in the control and vehicle control, respectively, and ranged from 0.101 to 0.113 g in the DMDS treatments. No treatment related effect on morphology or behaviour was identified. Water quality parameters were within acceptable limits throughout the exposure.

Applicant's summary and conclusion

Conclusions

Based on mean measured concentrations of DMDS, the NOEC and LOEC values for Fathead Minnow egg hatchability, total length, and blotted wet weight were 1.87 and >1.87 mg a.s./L, respectively. Based on mean measured concentrations of DMDS, the NOEC value for fry survival was 0.936 mg a.s./L.

Executive summary

The effect of the test item, Dimethyl disulphide (DMDS) on the fathead minnow (Pimephales promelas) embryos and fry during an early life-stage exposure was investigated under flow-through conditions, according to OECD 210 guideline. The test was conducted at nominal test concentrations of 0.13, 0.25, 0.50, 1.0, 2.0 and 4.0 mg/L. Due to loss of DMDS from volatility, analytical measured concentrations represented recoveries of 44 to 55% of nominal (mean measured concentrations were 0.0566, 0.122, 0.240, 0.549, 0.936 and 1.87 mg/L) and therefore the reported effect levels are based on mean measured concentrations.

Initiation of the study was to embryos <24 hours post fertilisation and sufficient embryos were used to give a group size of at least 15 embryos per replicate. There were 4 replicates for each treatment group. Day 0 post-hatch was based on ≥ 95% hatch in the control group. Day 0 was reach after 5 days incubation. All live fry were counted and released into their respective replicate growth chambers on day 7 post-hatch and exposure continued for 28 days post-hatch. Survival was monitored daily by visually inspecting each test chamber, and any behavioural or physical changes were recorded, including abnormalities. After 33 days exposure (including the egg incubation period) the fish were euthanised and total length and blotted weight were measured.

Hatching success in the control and vehicle control was 98 and 100%, respectively, and ranged from 95 to 100% in the DMDS treatments. Post-hatch survival of fry in the control and vehicle control was 94 and 91%, respectively, and ranged from 75 to 94% in the DMDS treatments. Mean total length was 23.3 and 23.0 mm in the control and vehicle control, respectively, and ranged from 21.6 to 22.9 mm in the DMDS treatments. Mean blotted wet weight was 0.101 and 0.107 g in the control and vehicle control, respectively, and ranged from 0.101 to 0.113 g in the DMDS treatments. Water quality parameters were within acceptable limits throughout the exposure. All validity criteria were met.

Based on geometric mean measured concentrations of DMDS, the NOEC and LOEC values for P. promelas egg

hatchability, total length, and blotted wet weight were 1.87 and >1.87 mg/L, respectively (i.e. no effect was seen on these endpoints in this study).
Based on geometric mean measured concentrations of DMDS, the NOEC was 0.94 mg/L for larval survival.

[Study 2] FELS sheepshead minnow, Gerke 2011/K1/Long-term toxicity to fish.001

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable without restrictions)	Gerke A.	2011	Dimethyl disulfide (DMDS): Early Life-Stage Toxicity Test with the Sheepshead Minnow, Cyprinodon variegatus, Under Flow-Through Conditions

Materials and methods

Life stage / endpoint studied : early-life stage: reproduction, (sub)lethal effects

Test guideline

Qualifier	Guideline	Deviations	GLP Compliance
according to	OECD Guideline 210 (Fish, Early-Life Stage Toxicity Test)		yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.7%

Analytical monitoring : yes

Details on analytical methods

Concentrations of DMDS were analytically verified using a validated HPLC/UV method. The concentrations of DMDS were measured in test solution samples (10 mL) collected two days prior to initiation (day -2) and on days 0, 7, 14, 21, 28, 35 and 38 (termination) of the definitive test. A single replicate sample was collected from the control, vehicle control, and test substance treatments; alternating replicates were sampled at each sampling event. The concentrations of DMDS in the diluter stock solutions were determined in samples collected on the same days as the test solutions.

Vehicle : yes

Details on test solutions

PREPARATION AND APPLICATION OF TEST SOLUTION
- Method:flow through
- Eluate: the Hamilton Model 420 syringe dispenser introduced 0.4-mL volumes of the diluter stock solution to the diluter system, where the diluter stock solution volume was diluted with approximately 3,990 mL of dilution water.
- Nominal concentrations of 0 (control), 0 (vehicle control), 0.065, 0.13, 0.25, 0.50, 1.0, and 2.0 mg a.i./L.
- Controls: The concentration of DMF in test solutions was 0.10 mL DMF/L. The control consisted of dilution water only.
- Chemical name of vehicle (organic solvent, emulsifier or dispersant): DMF, dimethylformamide
- Diluter stock solutions were prepared at a target concentration of 77.5 g a.i./L approximately every 7 - 11 days during the diluter equilibration and the definitive testing by diluting approximately 15.55 g of DMDS (15.5 g corrected for purity) to a volume of 0.200 L with dimethyl formamide (DMF).
- Evidence of undissolved material (e.g. precipitate, surface film, etc): All test solutions were clear and colorless with

no visible particulate material, surface film, undissolved test substance, or precipitate throughout the test.

Test organisms

Test organisms (species) : Cyprinodon variegatus

Details on test organisms

Species: Cyprinodon variegatus
Source: In-house culture
Age/Size: embryos were <24 hours post fertilisation
Holding: 25 ± 2ºC, light intensity 425-665 lux, under flow-through conditions, 10 volume additions to each test chamber over a 24 hour period were achieved.
Food/Feeding: Initially fry were fed live brine shrimp (Artemia sp.). A standard commercial fish food was added to the diet on study day 24, fish were fed live brine shrimp ad libitum once on study days 6 and 37 and 3 times daily during the remainder of the test. The fish were not fed for at least 24 hours prior to termination of the definitive test.

Study design

Test type : flow-through

Water media type: saltwater

Limit test : no

Total exposure duration : 38 d

Test conditions

Hardness : 142-154 mg CaCO3 /L

Test temperature : 24.2 to 25.5°C

pH : 6.8 to 8.1

Dissolved oxygen : 5.9 to 7.5 mg/L

Salinity : The dilution water was a laboratory saltwater prepared by adding a commercial sea salt mix (Crystal Sea Marinemix; Marine Enterprises International, Inc. Baltimore, Maryland) to laboratory freshwater at a target salinity 20 ± 3‰.

Nominal and measured concentrations

Nominal concentrations of 0 (control), 0 (vehicle control), 0.065, 0.13, 0.25, 0.50, 1.0, and 2.0 mg a.i./L. The mean measured DMDS concentrations in the test substance treatment solutions were 0.0288, 0.0615, 0.123, 0.229, 0.473, and 0.952 mg a.i./L, which represented recoveries of 44 to 49% of the nominal concentrations
Number of Replicates:4

Details on test conditions

The test was initiated on 19 July 2011 when five embryos were impartially distributed to the incubation cups suspended in each test chamber. The process was repeated until 20 embryos were present in each chamber. To account for any unsuspected losses due to fungus or other non-treatment related effects, the total initial number of embryos was greater than needed to satisfy the testing guidelines (i.e., 15 embryos per replicate). On a daily basis during incubation, the embryos were counted and dead embryos were removed and discarded when observed. There was no fungus observed during the definitive testing. When all living embryos had hatched, length of time to 95% hatch and hatchability were recorded. Day 0 post-hatch was based on ≥95% hatch in the control groups (i.e., control and vehicle control groups) which occurred on 29 July 2011 (study day 10). All live fry were counted and released into their respective replicate growth chambers on study day 16 (day 6 post-hatch). Survival was monitored daily by visually inspecting each test chamber, and any behavioral or physical changes were recorded, including abnormalities. After 28 days of post-hatch growth (study day 38), surviving fish were carefully netted from each replicate chamber and euthanized with tricaine methanesulfonate (MS-222; Argent Chemical Laboratories). All individuals were measured for standard length (i.e., tip of the snout to the caudal peduncle) using a millimeter scale and blotted wet weight using an electronic balance.

Reference substance (positive control): no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
38 d	NOEC	0.473 mg/L	meas. (geom. mean)	test mat.	other: hatchability and fry survival	
38 d	LOEC	0.952 mg/L	meas. (geom. mean)	test mat.	other: hatchability and fry survival	

Details on results

Loss of the test item resulting from volatility was observed, so the biological response results were reported based upon the mean measured DMDS concentrations.
In the control and vehicle control treatments, egg hatch began on day 6 and was completed on day 14. Complete hatch (≥95% hatch in the control and vehicle control treatments) was determined to be day 10 (day 0 post-hatch). Overall hatching success in the control and vehicle control treatments was 94 and 93%, respectively. Hatching success in the test substance treatments were 94, 94, 96, 93, 89, and 73% in the 0.0288, 0.0615, 0.123, 0.229, 0.473, and 0.952 mg a.i./L treatments, respectively. There was a statistically significant reduction in hatch success observed in the 0.952 mg a.i./L test substance treatment as compared to the control. Based on hatching success, the NOEC was 0.473 mg a.i./L.
Post-hatch survival was calculated as the percent of hatched fry that were alive at test termination (day 38; 28 days post-hatch). Post-hatch survival in the control and vehicle control treatment was 93 and 96%, respectively. Post-hatch survival in the test substance treatments were 93, 96, 99, 97, 93, and 50% in the 0.0288, 0.0615, 0.123, 0.229, 0.473, and 0.952 mg a.i./L treatments, respectively. There was a statistically significant reduction in post-hatch survival observed at the 0.952 mg a.i./L treatment levels, as compared to the control. Based on post-hatch survival, the NOEC was 0.473 mg a.i./L.
Growth of surviving fry was assessed at test termination (day 38; 28 days post-hatch) through standard length and blotted wet weight measurements. Replicate values were pooled into a treatment mean using the number of individuals present at the end of the test in weighting the means. Mean standard length in the control and vehicle control were 14.2 and 15.3 mm, respectively. Mean standard length in the test substance treatments were 15.2, 14.8, 14.6, 14.8, 13.7, and 12.3 mm in the 0.0288, 0.0615, 0.123, 0.229, 0.473, and 0.952 mg a.i./L treatments, respectively. The highest treatment level was removed from statistical analyses due to a statistically significant survival effect. There was no statistically significant reduction in standard length observed in any of the remaining treatment levels, as compared to the control. NOEC for standard length was 0.473 mg a.i./L.
Mean blotted wet weight in the control and vehicle control treatments was 0.094 and 0.125 grams, respectively. Mean blotted wet weight in the test substance treatments were 0.124, 0.117, 0.114, 0.119, 0.099, and 0.072 grams, respectively. The highest treatment level was removed from statistical analyses due to a statistically significant survival effect. There was no statistically significant reduction in blotted wet weight in any of the remaining treatment levels, as compared to the control. The NOEC for blotted wet weight was 0.473 mg a.i./L.

Applicant's summary and conclusion

Conclusions

Based on mean measured concentrations of Dimethyl Disulfide, the NOEC for both hatchability and fry survival ws 0.473 mg a.i./L, respectively. Based on mean measured concentrations of Dimethyl Disulfide, the NOEC and LOEC values for standard length and blotted wet weight were 0.473 mg a.i./L and >0.473 mg a.i./L, respectively.

Executive summary

The effect of the test item, Dimethyl disulphide (DMDS) on the sheepshead minnow (Cyprinodon variegatus) embryos and fry during an early life-stage exposure was investigated under flow-through conditions. The test was conducted at nominal test concentrations of 0.065, 0.13, 0.25, 0.50, 1.0, and 2.0 mg/L. Due to loss of DMDS from volatility, analytical measured concentrations represented recoveries of 44 to 55% of nominal (mean measured concentrations were 0.0288, 0.0615, 0.123, 0.229, 0.473, and 0.952 mg/L) and therefore the reported effect levels are based on geometric mean measured concentrations.
Initiation of the study was to embryos <24 hours post fertilisation and sufficient embryos were used to give a group size of at least 15 embryos per replicate. There were 4 replicates for each treatment group. Day 0 post-hatch was based on ≥95% hatch in the control group. Day 0 was reached after 10 days incubation. All live fry were counted and released

into their respective replicate growth chambers on day 7 post-hatch and exposure continued for 28 days post-hatch. Survival was monitored daily by visually inspecting each test chamber, and any behavioural or physical changes were recorded, including abnormalities. After 38 days exposure (including the egg incubation period), the fish were euthanasised and total length and blotted weight were measured.
Hatching success in the control and vehicle control was 94 and 93%, respectively, and ranged from 73 to 94% in the DMDS treatments. Post-hatch survival of fry in the control and vehicle control was 93 and 96%, respectively, and ranged from 50 to 99% in the DMDS treatments. Mean total length was 14.2 and 15.3 mm in the control and vehicle control, respectively, and ranged from 12.3 to 15.2 mm in the DMDS treatments. Mean blotted wet weight was 0.094 and 0.125 g in the control and vehicle control, respectively, and ranged from 0.072 to 0.124 g in the DMDS treatments. Water quality parameters were within acceptable limits throughout the exposure. All validity criteria were met.
Based on geometric mean measured concentrations of DMDS, the NOEC and LOEC values for fathead minnow egg hatchability, total length, and blotted wet weight were 1.87 and >1.87 mg a.i./L, respectively (i.e. no effect was seen on these endpoints in this study).
Based on mean measured concentrations of DMDS, the NOEC for larval survival was 0.94 mg/L.

4.5.2 Chronic toxicity to aquatic invertebrates

[Study 1] daphnia repro_2011/K1 KS/Long-term toxicity to aquatic invertebrates.001

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable without restrictions)	Rebstock M.	2011	Dimethyl Disulfide (DMDS): Chronic Toxicity Test with the Water Flea, Daphnia magna, Conducted Under Static-Renewal Test Conditions

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 211 (Daphnia magna Reproduction Test)	

GLP compliance: yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.7%

Analytical monitoring : yes

Details on sampling

The concentrations of DMDS were measured in fresh test solutions collected from parent solutions on days 0, 6, 13, and 20 and corresponding spent test solutions which were collected after compositing replicate solutions by treatment on days 1, 7, 14, and 21. The working standard solutions used to prepare test solutions were sampled and analyzed along with each set of fresh test solutions on day 0, 6, 13, and 20 as well as with the spent solutions on day 1. Additional samples of the working standard solutions used to prepare the 0.0013, 0.0025, and 0.0050 mg/L test solutions were collected one day after the termination of the definitive test due to a dilution error during the preparation of the corresponding dosing solutions on day 20. A sample volume of approximate 10 mL was collected from the controls and each test substance treatment. Due to the instrument sensitivity limitations of the analytical method, samples were not diluted prior to being vialed for analysis by HPLC-UV.

Details on analytical methods

HPLC-UV: The analytical method was validated in the fish ELS study (Rebstock, M. 2011, Dimethyl disulfide (DMDS): Early Life-Stage Toxicity Test with the Fathead Minnow, Pimephales promelas, Under Flow-Through Conditions; Report Number (Study) 66381). The calibration curve for each analysis ranged from 0.00584 to 0.0730 mg/L and the validated limit of quantification was 0.0139 mg/L (lowest concentration where accuracy and precision was demonstrated in the validation study).

Vehicle : yes

Details on test solutions

Working standard solutions were prepared by serially diluting a 1.6 mg a.i./mL stock solution (dissolved in DMF) to concentrations of 0.052, 0.10, 0.20, 0.40, and 0.80 mg a.i./mL. Test solutions were prepared by diluting 0.050-mL volumes of the appropriate working standard to a volume of 2.0 L with dilution water, which resulted in nominal solution test concentrations of 0.0013, 0.0025, 0.0050, 0.010, and 0.020 mg a.i./L. The vehicle control was prepared by diluting 0.050 mL of DMF to a volume of 2.0 L with dilution water. The concentration of DMF in the vehicle control and the treated test solutions was 0.025 mL DMF/L. The control solution consisted only of dilution water.

Test organisms

Test organisms (species) : Daphnia magna

Details on test organisms

- Source: In-house culture
- Culture: All daphnids were cultured at approximately 20°C during the holding period, fed a suspension of the algal species Pseudokirchneriella subcapitata at least once a day supplemented by a prepared artificial diet consisting of a wheat grass, salmon starter, and yeast suspension. Approximately one day prior to neonate selection, the adult daphnids were isolated by transferring the adults to a fresh culture with a water/food suspension. The adults were considered acceptable with no signs of stress, disease or physical damage. No adult mortality occurred during the 48-hour period immediately prior to production of neonates used in test initiation.
- Acclimation: Since the culturing and testing environmental parameters were equivalent (i.e., temperature, dilution water, and lighting), no acclimation period was necessary.
- Feeding: The daphnids were fed daily a diet consisting of 1.0 mL of a 3.0 x 10^7 cells/mL concentrated algal suspension (Pseudokirchneriella subcapitata, formerly Selenastrum capricornutum) and 0.5 mL of a 1.08 g/L or 1.71 g/L YTC daphnid feed mixture.
- Age of Test Organisms at start of the Test: <24 h

Study design

Test type : semi-static

Water media type: freshwater

Limit test : no

Total exposure duration : 21 d

Test conditions

Hardness : 142-146 mg CaCO3/L

Test temperature : 20.0 - 20.9

pH : 8.1 - 8.5

Dissolved oxygen :7.0 - 8.7

Salinity : not relevant

Nominal and measured concentrations

Nominal concentrations: 0 (control), 0 (vehicle control), 0.0013, 0.0025, 0.0050, 0.010, and 0.020 mg a.s./L. In

accordance with OECD guidance n°23 on aquatic toxicity testing with difficult substances (p. 43): "*for tests with chemicals that cannot be quantified by analytical methods at the concentrations causing effects, the effect concentration can be expressed based on the nominal concentrations*", the results were based on nominal DMDS concentrations since the test concentrations were outside the range of the analytical method, with the exception of the top test concentration of 0.020 mg/L DMDS. All technical efforts were made to get LOQ/LOD as low as technically possible when developing the analytical method.

Details on test conditions

The test vessels were 125 mL glass jars sealed with PTFE lined screw on lids. The jars for each test were filled with the appropriate volume of control, vehicle control, or test substance solution so as to minimize headspace within the sealed vessel. The test chambers were maintained at 20 ± 1°C in a temperature-controlled water bath. Fluorescent lighting was maintained on a 16-hour daylight photoperiod with two 30 minute simulated dawn and dusk periods. The light intensity during the definitive test was 561 lux as measured on Day 0. Observations were made daily on the number of surviving adult daphnids, occurrence of abnormalities, and production of neonates. Immobile daphnids, defined as those organisms not able to swim within 15 seconds after gentle agitation of the test vessel or gentle disturbance of the individual, were discarded; therefore, immobility was synonymous with mortality. Each day, the adult daphnids (i.e., parental generation) were transferred to clean test chambers containing freshly prepared test solutions. After the release of broods by the adult daphnids, only the adult daphnids were transferred to fresh test solutions. The neonates produced between each renewal and at termination were counted and discarded.At test termination, the length (head to base of spine) of each surviving adult was measured. Mean adult dry weight for the controls and each test substance treatment was determined after combining the surviving adult daphnids by treatment and drying the samples at approximately 60°C for approximately 48 hours.

Reference substance (positive control) : no

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
21 d	NOEC	0.0025 mg/L	nominal	test mat.	reproduction	
21 d	LOEC	0.005 mg/L	nominal	test mat.	reproduction	

Details on results

The total numbers of live young produced by surviving first generation daphnids after a 21-day exposure were 180, 177, 178, 174, 154, 159, and 150 in the 0 (control), 0 (vehicle control), 0.0013, 0.0025, 0.0050, 0.010, and 0.020 mg a.i./L treatments, respectively. Based on the total numbers of live young produced per surviving first generation daphnids, the 21-day NOEC and LOEC were 0.0025 and 0.0050 mg a.i./L, respectively. Mean day of first brood release were 8, 8, 8, 8, 8, 10, and 8 in the 0 (control), 0 (vehicle control), 0.0013, 0.0025, 0.0050, 0.010, and 0.020 mg a.i./L treatments, respectively. There was a significant increase in days to first brood release in the 0.010 mg a.i./L treatment compared to the control. However, this statistically significant difference was not considered to be a concentration dependent response and was therefore considered not relevant. Based on days to first brood, the 21-day NOEC and LOEC values were 0.020 and >0.020 mg a.i./L, respectively. Individual lengths (helmet-to-spine) of all surviving parent daphnids at day 21 was measured. Mean length of the daphnids were 4.6, 4.6, 4.5, 4.4, 4.4, 4.4, and 4.2 mm for the 0 (control), 0 (vehicle control), 0.0013, 0.0025, 0.0050, 0.010, and 0.020 mg a.i./L treatments, respectively. Based on length, the 21-day NOEC and LOEC were 0.010 and 0.020 mg a.i./L, respectively.

Applicant's summary and conclusion

Conclusions

The test acceptability criteria were met for this study. The mortality of the parent animals in the control did not exceed 20% at the end of the test. The mean number of live young produced per surviving adult daphnid at the end of the test was ≥60 in the control group. This study is considered acceptable and satisfies the guideline requirement for a reproduction test with Daphnia magna. Total young per surviving adult was the most sensitive biological parameter. The 21-day NOEC was 0.0025 mg/L.

Executive summary

A freshwater 21 day static-renewal chronic toxicity test with Daphnia magna was performed according to OECD Guideline 211 to assess the toxicity of dimethyldisulfide (DMDS) at nominal test concentrations of 0 (control), 0 (solvent control), 0.0013, 0.0025, 0.0050, 0.010, and 0.020 mg/L (concentrations selected on the basis of the results of a range finding test). The definitive test was conducted for 21 days commencing when daphnids (<24 hours old at the start of the test) were added to the test chambers. 10 replicates (1 daphnid per test container) were included and the daphnids were fed daily. Each day the parental generation daphnids were transferred to clean test chambers containing freshly prepared test solutions. Observations were made daily on the number of surviving adult daphnids, occurrence of abnormalities, and production of neonates. The neonates produced between each renewal and at termination were counted and discarded. At test termination, the length of each surviving adult was measured and the mean adult dry weight for the controls and each test substance treatment was determined after combining the surviving adult daphnids by treatment. Water quality measurements and water temperature were monitored throughout the test. The results are based on nominal concentrations of DMDS since the test concentrations (in both fresh and expired media) were outside the range of the analytical method, with the exception of the top test concentration. This is in accordance with OECD guidance n°23 on aquatic toxicity testing with difficult substances (p. 43): "*for tests with chemicals that cannot be quantified by analytical methods at the concentrations causing effects, the effect concentration can be expressed based on the nominal concentrations"*. All technical efforts were made to get LOQ/LOD as low as technically possible when developing the analytical method.
Statistical analysis was performed to assess the effects of DMDS on adult survival, reproduction and adult length. There was no significant reduction in survival in any test treatment as compared to the dilution water control survival. Based on parent survival, the 21-day NOEC and LOEC were 0.020 and >0.020 mg/L, respectively (i.e., no effect was seen on this endpoint in this study). Regarding parental length at the end of the study, the 21-day NOEC was 0.01 mg/L. Based on the total numbers of live young produced per surviving parent daphnids, the 21-day NOEC was 0.0025 mg/L.

[Study 2] Americamysis bahia Life cycle_2011/K1 KS/ Long-term toxicity to aquatic invertebrates.002

Study Reference

Reference type	Purpose Flag	Reliability	Author	Year	Title
study report	Key study	1 (reliable without restrictions)	Gerke A.	2011	Dimethyl Disulfide (DMDS): Life-Cycle Toxicity Test of the Saltwater Mysid, Americamysis bahia, Conducted under Flow-Through Conditions

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	EPA OPPTS 850.1350 (Mysid Chronic Toxicity Test)	

GLP compliance : yes (incl. certificate)

Test materials

Test material used in the study equivalent to the substance identified in the C&L dossier
Purity: 99.7%

Analytical monitoring : yes

Details on sampling

A sample volume of approximate 10 mL was collected from the controls and each test substance treatment. A 0.020 mL sample was collected from the diluter stock solution. Samples were diluted with freshwater, if necessary, to produce sample concentrations that fell within the calibration curve (0.0219 to 0.730 mg a.i./L). The samples were vialed for analysis by HPLC/UV.

Details on analytical methods

- HPLC/UVInstrument: Hewlett Packard HPLC system equipped with an UV detector
- Column: YMC pack pro C18, 150 mm x 4.6 mm x 3µm
- Column Temp.: 40°C
- Mobile Phase (Isocratic): 40:60 acetonitrile:0.1% phosphoric acid
- Flow Rate: 1.2 mL/minute
- Injection Volume: 50 µL
- Run Time: 10 minutes
- Wavelength: 200 nm

Vehicle : yes

Details on test solutions

The testing was performed at nominal concentrations of 0 (dilution water control), 0 (vehicle control), 0.25, 0.50, 1.0, 2.0, and 4.0 mg a.i./L. Diluter stock solutions were prepared at a target concentration of 156 mg/L by diluting approximately 15.6469 or 39.1174 g (15.6000 or 39.0000 g corrected for purity, respectively) of dimethyl disulfide to a volume of 0.100 or 0.250 L, respectively, with dimethylformamide (DMF). Diluter stock solutions were prepared in this manner on 13, 23, and 31 August 2011, and on 06 and 12 September 2011. All diluter stock solutions were stored covered and ventilated at room temperature.

Test organisms

Test organisms (species) : Americamysis bahia

Details on test organisms

Mysids (Americamysis bahia) were obtained from in-house cultures that were maintained at ABC Laboratories, Inc. The adult mysids used to generate the juvenile test organisms were cultured in artificial saltwater with salinity of approximately 20‰ and at a temperature of approximately 25°C. The animals were fed brine shrimp nauplii (Artemia sp. <48 hours old). Juvenile mysids used for the definitive test were collected <24 hours old at test initiation. The mysids were fed live brine shrimp nauplii (Artemia sp.) at least twice daily during the study.

Study design

Test type : flow-through

Water media type : saltwater

Total exposure duration : 28 d

Test conditions

Hardness : not relevant

Test temperature : 24.1 - 24.9 °C

pH : 7.7 - 8.1

Dissolved oxygen : from 3.8 to 7.0 mg/L or 52 to 97% saturation

Salinity : Laboratory saltwater prepared by adding a commercial sea salt mix (Crystal Sea Marinemix; Marine Enterprises International, Inc. Baltimore, Maryland) to laboratory freshwater at a target salinity 20 ± 2‰. The laboratory freshwater consists of well water blended with well water that was demineralized by reverse osmosis.

Nominal and measured concentrations : see below

Reference substance (positive control) : no

Any other information on materials and methods incl. tables

Due to the volatile nature of the test substance, analytical samples were measured from both the F0 and F1 exposure sides of the test chambers. Measured concentrations of dimethyl disulfide in the test substance treatment solutions prior to initiation (day -2) were 0.131, 0.259, 0.450, 0.740, and 1.62 mg a.i./L, which represented recoveries of 37 to 52% of the nominal concentrations. The DMDS measured concentrations at initiation (0-hour) from the F0 exposure side were 0.119, 0.268, 0.468, 0.818, and 1.73 mg a.i./L, or 41 to 54% of the nominal concentrations. The DMDS measured concentrations on day 7 from the F0 exposure side were 0.112, 0.192, 0.410, 0.900, and 1.62 mg a.i./L, or 38 to 45% of the nominal concentrations. The measured DMDS concentrations ranged from 26 to 54% in the F1 exposure side treatments and from 27 to 63% in the F0 exposure side treatments on days 14, 21, and 28. Measured DMDS concentrations in the diluter stock solutions ranged from 92 to 97% of the nominal concentration. No residues of DMDS were detected in the control or vehicle control solutions above the MQL for the analyte during the exposure period. The mean measured DMDS concentrations in the test substance treatment solutions for the F0 exposure were 0.125, 0.250, 0.464, 0.900, and 1.73 mg a.i./L, which represented recoveries of 43 to 50% of the nominal concentrations. The mean measured DMDS concentrations in the test substance treatment solutions for the F1 exposure were 0.113, 0.227, 0.406, 0.861, and 1.68 mg a.i./L, which represented recoveries of 41 to 45% of the nominal concentrations. Recoveries from QC fortifications ranged from 90 to 105% of the nominal concentrations. There were 14 instances where the measured DMDS concentrations deviated by >20% from the mean measured concentrations. Since there were only 14 instances during the entire definitive test, and there was not a systematic deviation (e.g., one specific level of exposure or a trend along time), we consider that this does not prevent conclusions of the toxicity of DMDS from this study. The biological response results were reported based upon the F1 or F0 mean measured DMDS concentrations as appropriate for the biological endpoint.

Results and discussions

Effect concentrations

Duration	**Endpoint**	**Effect conc.**	**Nominal/Measured**	**Conc. based on**	**Basis for effect**	**Remarks (e.g. 95% CL)**
28 d	NOEC	0.464 mg/L	meas. (geom. mean)	test mat.	other: mean total young per surviving pair	
28 d	LOEC	0.9 mg/L	meas. (geom. mean)	test mat.	other: Mean total young per surviving pair	

Details on results

Mean number of young per female was the most sensitive parameter. Other parameters are reported in the Executive summary

Applicant's summary and conclusion

Conclusions

The test acceptability criteria for this study were met. The water-quality characteristics remained within the tolerance limits set forth in the protocol. Survival of the dilution water control and vehicle control F0 mysids was 98%. The percentage of dilution water control and vehicle control F0 female mysids available to produce young that actually did produce a brood were 100 and 95%, respectively. The average total of young produced per dilution water control and vehicle control F0 female mysid were 16.0 and 11.9, respectively and the average number of young per brood release was greater than three for both control treatments.

Executive summary

A life-cycle toxicity test under flow-through conditions with the saltwater mysid, Americamysis bahia, exposed to dimethyldisulfide (DMDS) was performed according to OPPTS 850.1350. The purpose of this test was to assess test substance effects on survival, growth, and reproduction of mysids, and to determine a no-observed-effect concentration (NOEC). Comparisons between control and exposure treatments were made to determine the test substance concentrations at which statistically significant reductions in the measured parameters occurred.
Based on mean measured concentrations of dimethyldisulfide during the 28-day exposure, the F0 mysid survival LC50 was >1.73 mg/L, and the NOEC was 1.73 mg/L, the highest concentration tested (i.e., no effect was seen on this endpoint during the study). The NOEC for F0 male and female mysid length on day 14, and female mysid length on day 28 was 1.73 mg/L, the highest concentration tested (i.e., no effect was seen on these endpoints during the study). The NOEC for F0 male mysid length on day 28 was 0.9 mg/L. The NOEC for F0 mysid day of first brood and mean number of total young produced per female were 0.9 and 0.46 mg/L, respectively. The NOEC for F1 mysid survival on day 10

was 0.861 mg/L, the highest concentration tested (i.e., no effect was seen on this endpoint during the study). The NOEC for F1 male and female mysid length on day 10 was 0.861 mg/L.