Committee for Risk Assessment

RAC

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

Adopted

8 June 2018

8 June 2018

CLH-O-0000001412-86-218/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **Dimethyl disulphide**

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

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

The proposal was submitted by **Arkema France** and received by RAC on **16 May 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Arkema France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **23 May 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **7 July 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

Co-Rapporteur, appointed by RAC: **Steven Dungey**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 June 2018** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	TBD	Dimethyl disulphide	210-871-0	624-92-0	Flam. Liq. 2 Acute Tox. 4 Acute Tox. 3 Eye Irrit. 2 Skin Sens. 1B 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 =1 M =10	
RAC opinion	TBD	Dimethyl disulphide	210-871-0	624-92-0	Flam. Liq. 2 Acute Tox. 3 Acute Tox. 3 STOT SE 3 STOT SE 1 Eye Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H225 H301 H331 H336 H370 (upper respiratory tract, inhalation) H319 H317 H400 H410	GHS02 GHS06 GHS08 GHS09 Dgr	H225 H301 H331 H336 H370 (upper respiratory tract, inhalation) H319 H317 H410		oral: ATE = 190 mg/kg bw inhalation: ATE = 5 mg/L M =1 M =10	
Resulting Annex VI entry if agreed by COM	TBD	Dimethyl disulphide	210-871-0	624-92-0	Flam. Liq. 2 Acute Tox. 3 Acute Tox. 3 STOT SE 3 STOT SE 1 Eye Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H225 H301 H331 H336 H370 (upper respiratory tract, inhalation) H319 H317 H400 H410	GHS02 GHS06 GHS08 GHS09 Dgr	H225 H301 H331 H336 H370 (upper respiratory tract, inhalation) H319 H317 H410		oral: ATE = 190 mg/kg bw inhalation: ATE = 5 mg/L M = 1 M = 10	

HUMAN HEALTH HAZARD EVALUATION

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 ($300 < LD_{50} \leq 2000$ 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 ($LD_{50} \leq 2000$ 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 ($2.0 < LC_{50} \leq 10.0$ 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.

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)**.

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**.

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)**.

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)**.

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 ≤ 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 considers that data are conclusive, but **not sufficient for classification for (STOT RE)**.

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**.

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 (%), mean	79	81	83	83
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 did not deliver	3	1	2	1
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 of pups born	14.7	15.1	14.6	15.3
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 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

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**.

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 mm^2/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.**

ENVIRONMENTAL HAZARD EVALUATION

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	*Americamysis bahia* (mysid shrimp)	28-d $NOEC_{repro}$	0.464	Gerke, 2011b

Method	Test organism	Endpoint	Toxicity values in mg a.s./L	Reference
Short-term toxicity to fish				
(flow-through)				
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 mean concentrations)	Scheerbaum, 2007c
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.

Additional references

J Appl Toxicol. 1988 Apr;8(2):91-4

Teratog Carcinog Mutagen. 1986;6(5):419-29

J Appl Toxicol. 1988 Apr;8(2):91-4

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).