Committee for Risk Assessment

RAC

Annex 1

Background document

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

6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol; [DBMC]

EC Number: 204-327-1
CAS Number: 119-47-1

CLH-O-0000001412-86-288/F

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

Adopted

13 June 2019

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (DBMC)

EC Number: 204-327-1

CAS Number: 119-47-1

Index Number: -

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Version number: 2 **Date: 31 July 2018**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 6,6'-DI-*TERT*-BUTYL-2,2'-METHYLENEDI-*P*-CRESOL (DBMC)

Note on confidential information

Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information. Such information should be provided in a separate confidential Annex to this report, clearly marked as such.

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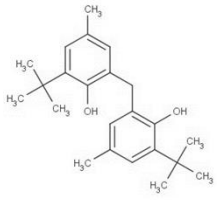
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 6,6'-DI-*TERT*-BUTYL-2,2'-METHYLENEDI-*P*-CRESOL (DBMC)

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol
Other names (usual name, trade name, abbreviation)	DBMC; Phenol,2,2'-methylenebis-(6-(1,1-dimethylethyl))-4-methyl-; 6,6'-di-tert-butyl-4,4'-dimethyl-2,2'-methylenediphenol; 2,2'methylenebis(4-methyl-6-tert-butylphenol) 2,2'-methylenebis(6-*tert*-butyl-*p*-cresol); bis(6-hydroxy-3-methyl-5-tert-butylphenyl)methane Vulkanox BKF; Antioxidant 2246; Ionol 46 Lowinox 22M46 Ralox 46 SANTOWHITE PC ANTIOXIDANT
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	204-327-1
EC name (if available and appropriate)	6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol
CAS number (if available)	119-47-1
Other identity code (if available)	
Molecular formula	$C_{23}H_{32}O_2$
Structural formula	
SMILES notation (if available)	Oc(c(cc(c1)C)Cc(c(O)c(cc2C)C(C)(C)C)c2)c1C(C)(C)C
Molecular weight or molecular weight range	340.51g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	n.a.
Description of the manufacturing process and identity of the source (for UVCB substances only)	n.a.

Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant for classification

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (DBMC) EC Number: 204-327-1 CAS no: 119-47-1			

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Not relevant for classification				

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not relevant for classification					

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

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (DBMC) EC Number: 204-327-1 CAS no: 119-47-1	**Not available in 4 studies; 91.8% in 2 studies; 96-98%; >98%; >99%**	**Some impurities given for study 7 and 9, otherwise not.**		

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	Not listed in Annex VI	-	-	-	-	-	-	-	-	-	-
Dossier submitters proposal			**204-327-1**	**119-47-1**	Repr. 1B	H360F	GHS08 Dng	H360F		-	
Resulting Annex VI entry if agreed by RAC and COM			**204-327-1**	**119-47-1**	Repr. 1B	H360F	GHS08 Dng	H360F			

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

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

No harmonised classification for this substance was previously discussed at EU-level.

RAC General comment
DBMC is an antioxidant and a stabilising additive used at industrial sites in manufacturing as well as by professional workers and by consumers. It does not have a previous entry in Annex VI of CLP. As the dossier submitter (DS) considered that the CLP criteria for classification as toxic to reproduction were fulfilled, a CLH proposal was submitted in accordance with CLP article 36(1)(d).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

As the substance 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (hereafter referred to as DBMC) is considered to fulfil the criteria for classification as toxic to reproduction (Repr. 1B) a harmonised classification is justified according to CLP article 36(1)(d).

Further detail on need of action at Community level

The Danish CA has performed a substance evaluation on DBMC, which was finalised with the issuing of a Conclusion Document on 30 June 2017. Based on the evaluation of the end point of reproductive toxicity, it was concluded that the evidence supported classification as toxic to reproduction in category 1B for effects on fertility, whilst the registration dossier included a category 2 classification for that end-point. The Danish CA thus disagrees with the registrant's self-classification. According to the Classification and Labelling Inventory 13 notifications, covering 265 notifiers, have self-classified DBMC as Repr. 2 whilst 10 notifications, covering 229 notifiers, did not classify the substance as toxic to reproduction (C&L inventory search, December 2017).

According to article 36(1)(d) of the CLP Regulation, substances fulfilling the criteria for reproductive toxicity should normally be subject to harmonised classification.

The Danish CA considers that regulatory actions are needed to reduce the risk for humans due to the serious effect of this substance on fertility. A classification as a reproductive toxicant in category 1B will automatically lead to risk management through REACH (e.g. REACH Annex XIV restriction of use in consumer products) and will provide the basis for further risk management actions such as nomination for the candidate list or elaboration of a restriction proposal if considered appropriate. Other down-stream regulation, e.g. through OSH legislation, will also be a direct consequence of a harmonised Repr. 1B classification. Voluntary reduced marketing and use of the substance is also likely to occur. These initiatives would increase protection of workers, consumers and the environment from exposure to the substance.

A testing proposal for a two-generation reproductive toxicity study (OECD TG 416) was proposed by the lead registrant under REACH due to a data gap in the registration dossier in 2011. This proposal was amended in April 2018 to include the extended one-generation reproductive toxicity study (EOGRTS) (OECD TG 443) instead, as the standard information requirement in the meantime has been amended to exchange OECD 416 for OECD 443. The testing proposal process is still ongoing.

The registrants state in their reference to the testing proposal for an EOGRTS under the end-point on reproductive toxicity of the registration dossier (ECHA dissemination site, visited July 2018): "Due to animal protection reasons the decision on the testing proposal EOGRTS OECD 443 should not be taken before the evaluation of the proposal for a harmonized classification of DBMC as toxic for reproduction category 1 B initiated by the Danish Authority is completed."

The registrants have "provisionally" classified the substance as reproductive toxic in category 2, as stated in their justification for classification/non classification (ECHA dissemination site, visited July 2018). Under the endpoint summary for reproductive toxicity the lead registrant states : ".The registrants further state: "Based on the recommendation given by regulation EC No 1907/2006 an Extended One-Generation Reproduction Toxicity study is proposed to be conducted and provisionally the test substance is classified in category 2 according to regulation (EG) 1272/2008 CLP). Final classification would be discussed depending on effects observed in the EOGRTS." (ECHA dissemination site, visited July 2018).

The dossier submitter for the classification proposal does not consider that the performance of an EOGRTS is necessary in order to conclude on the classification of DBMC as reproductive toxic for sexual function and fertility, as the available data consistently demonstrate testes and sperm effects in several studies and thus fulfil the criteria for classification in category 1B.

Should a harmonised classification of DBMC as reproduction toxicant in category 1B be agreed, it could potentially trigger adaptation possibilities under REACH, Annex X, 8.7, column 2, and further animal testing may thus be avoided.

5 IDENTIFIED USES

DBMC is manufactured in and/or imported into the European Economic Area in 1,000-10,000 tonnes per year. The substance is used at industrial sites in manufacturing as well as by professional workers and by consumers. It is an antioxidant and a stabilising additive.

Industrial and professional applications include rubber and non-rubber polymers, fuels, hydraulic and metal working fluids, adhesives, process regulators and in laboratory chemicals.

Consumer applications include fuels, lubricants and greases, metal working fluids, adhesives and sealants (e.g. in, floor coverings), paints and coatings, paper products and plastic and rubber products. (public registration, ECHA; Pubmed: https://pubchem.ncbi.nlm.nih.gov/compound/119-47-1#section=Top).

6 DATA SOURCES

Registered data information ECHA webpage (retrieved in November, 2017): https://echa.europa.eu/da/registration-dossier/-/registered-dossier/13380/3/1/4

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid: a white solid powder with a faint odour		

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Property	Value	Reference	Comment (e.g. measured or estimated)
Melting/freezing point	123 - 132°C	OECD SIDS, 2000; MITI, 1992; Lide, 2009; PBT Working Group, 2009; ChemIDPlus, 2009, JE/NC, 1982	Range from the 6 references is given. Weight-of-evidence.
Boiling point	185 - 187°C at 0.07 hPa	PBT Working Group, 2009; OECD SIDS, 2000	DBMC decomposes at 200 - 220 °C at atmospheric pressure (SFOS report, 1982).
Relative density	1.04 - 1.10 g/L	OECD SIDS, 2000; Ashford, 2001;	
Vapour pressure	4.6E-7 Pa at 20°C		Calculated by QSARs (highest value). Measured values only available for elevated temperatures 26.6 Pa at 150°C (SFOS), and 53.33 Pa at 175°C)
Surface tension	Not applicable		water solubility < 1 mg/L at 20 °C
Water solubility	0.007 mg/L at 20ºC	Neuland, 2010	Column elution method and HPLC-analysis according to OECD TG 105.
Partition coefficient n-octanol/water	Log Kow (Pow): 6.25 at 20ºC	CITI Japan, 1992; Bayer, 1989; Keller, 2010	Shake-flask method
Flash point	185 °C at 1013 hPa	Csaftari, 2010	
Flammability	n.a.		No functional groups with pyrophoric properties and not ignition in contact with water.
Explosive properties	Non explosive		DBMC is a solid with no explosive properties.
Self-ignition temperature	Not applicable	Klein, Blank, 2008	DBMC is a solid with a melting point < 160 °C. DBMC is not self-igniting EC method A.16 .
Oxidising properties	No		DBMC contains no oxidising groups.
Granulometry	Value used for CSA: Up to 75 % of particles < 10 µm	Starck, 2010	
Stability in organic solvents and identity of relevant degradation products	End point waived.		
Dissociation constant	pKa1: 11.55 at 20 ºC	.	QSAR programme SPARC v4.5
Viscosity	Not applicable		DBMC is a solid.

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

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

No studies on toxicokinetics of DBMC are available.

10 EVALUATION OF HEALTH HAZARDS

The acute toxicity data are not assessed in details. However, information from the disseminated registration dossier indicates that acute toxicity of DBMC is low, with $LD50_{oral}$ values greater than or even to 5000 mg/kg bw based on studies available in the registration dossier performed between 1952 to 1994 (Takagi 1994, American Cyanamid Company 1965, Hagan 1952, Garlanda 1962, Stasenkova 1977).

10.1 Acute toxicity - oral route

Not assessed.

10.2 Acute toxicity - dermal route

Not assessed.

10.3 Acute toxicity - inhalation route

Not assessed.

10.4 Skin corrosion/irritation

Not assessed.

10.5 Serious eye damage/eye irritation

Not assessed.

10.6 Respiratory sensitisation

Not assessed.

10.7 Skin sensitisation

Not assessed.

10.8 Germ cell mutagenicity

Not assessed.

10.9 Carcinogenicity

Not assessed.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Data relevant for this end-point were retrieved from the study reports and publications referenced in the REACH registration dossier under the end-point of reproductive toxicity, supplemented by the OECD SIDS report (OECD, 2003). A search in Pubmed did not yield any additional information regarding adverse effects on sexual function and fertility of DBMC, but a few studies on structural analogues were found. The collection of information is further elaborated and the information used in the Weight of Evidence analysis, presented in Annex II.

The registration dossier included results from one reproductive/developmental toxicity screening study in rats and several repeated dose toxicity studies. In these, male reproductive endpoints (testes histopathology and/or sperm parameters) were investigated in six studies in rats, one mouse study and one dog study. Exposure duration ranged from 28 days to 18 months.

The available studies relevant for assessment of effects on sexual function and fertility are summarised below in table 9. Detailed summaries of the studies are included in Annex I of this report. The study numbers also refer to study numbers used in Annex I and II.

The effects reported in table 9 below were statistically significant unless otherwise specified.

Table 9: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Study 1: Reproduction / Developmental Toxicity Screening Test, OECD Guideline (similar to OECD TG 421, although TG number not stated in the report), GLP	Test substance: DBMC, CAS no: 119-47-1 Screening oral: gavage 0, 12.5, 50, 200, 800 mg/kg bw/day (nominal) Exposure: Daily administration. male: 50-52 d, female: 40-48 d	**NOAEL:** Male: 12.5 mg/kg bw/day, Female: 50 mg/kg bw/day **LOAEL:** Male: 50 mg/kg bw/day, Female: 200 mg/kg bw/day **12.5 mg/kg bw/day:** No adverse effects in males or females were reported. **50 mg/kg bw/day:** Males: No effect on body weights or food consumption was seen during the study. At necropsy no effect on testes and epididymis weights were recorded, but giant cell formation in the testes (2/12 animals, slight grade) was present, however, not significantly different from the control group. Furthermore, statistically significant adverse effects on sperm quality	Ministry of Health and Welfare Japan (1999b)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
rat (Crj: CD(SD)) male/female n=12/sex Reliability: Klimish score 1	(from 14 days before mating to the day 3 of lactation) (daily)	were seen, including a 16 % decrease in sperm motility ratio, a 10 % decrease in sperm viability ratio, a 20 % decrease in sperm survivability ratio and a ~30 % decrease in number of sperm in the epididymis cauda. Also a 5 times increase in the abnormal sperm ratio was observed. Females: No adverse effects were noted, including no decrease in body weights. No effects on ovary weights or any adverse effects on fertility were seen. **200 mg/kg bw/day**: Males: There was no effect on body weight or food consumption seen during the dosing period. At necropsy no significant effects on body weight were seen. Absolute and relative testes weights were significantly reduced by ~ 16 % and absolute and relative epididymis weights were significantly reduced by ~ 12 %. Histopathological effects on the testes were seen, including significantly more animals with atrophy of the seminiferous tubules (6/12 animals, slight to marked grade), and a non-significant increase in animals with giant cell formation in the testes (2/12 animals, slight grade) was seen. Furthermore a significant decrease in sperm in the epididymis was seen (9/12 animals, slight to marked grade). The adverse effects on sperm were very severe and included an increase in abnormal sperm ratio (from 1.55 % in control to 56.3 % in this dose group), a ~80 % decrease in the sperm motility ratio, a 26 % decrease in sperm viability ratio, ~50 % decrease in sperm survivability ratio, and ~66 % decrease in number of sperm in the epididymis cauda. Females: Body weights gains were non-significantly reduced until delivery compared to controls. This effect became significant in the lactation period (day 1-4), and dam body weights were significantly decreased (by 7 %) at PND 4. Additionally, significantly lower food consumption was seen periodically during pre-mating, pregnancy and lactation. At necropsy on PND 4, absolute and relative ovary weights were not significantly affected, and neither were the number of corpora lutea and implantation scars. 8**00 mg/kg bw/day:** Males: During the dosing period, no effect on body weight was seen, but a transient decrease in food consumption (one day in the beginning of the dosing period) was observed. At necropsy no effect on body weight was noted. Absolute and relative testes weights were decreased by ~ 50 %, and a	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		~25 % decrease was seen in absolute and relative epididymis weights. Histological examination showed severe effects on the testes, including atrophy of seminiferous tubules (12/12 animals, mild to marked grade), and a severe decrease in sperm was seen in the epididymis (12/12 animals, marked grade). No motile sperm was evident in any of the 12 examined males, and the number of abnormal sperm was very high. Moreover, the total number of sperm in cauda epididymis was ~80 % decreased, compared to control males. Females: Body weights were not affected during premating, but were significantly lower than those of the controls during pregnancy (6% and 8% on PD 21 with and without correction for pup weights, respectively) and (9 %, corrected) at study termination. Body weight gain was depressed (29%) from study start until termination compared to controls. Food consumption was periodically lower during pre-mating, pregnancy and lactation, and occasional cases of loose stools and salivation were reported (9/12 animals normal). There were no effects on ovary weights, but a significant 14 % decrease in the number of corporae luteae and a significant 8 % decrease in the number of implantation scars were seen.	
Study 2: Sub-chronic oral toxicity study, not guideline, not GLP Rat (Wistar) male/female, in total n=10/sex/dose, 5/sex /dose for each exposure period (4 and 12 weeks) Body weight and food consumption were recorded weekly, and general condition were observed daily. Haematological and serum	Test substance: DBMC CAS no. 119-47-1, Subchronic, oral: Dietary levels of 0, 1200, 6000, 30.000 ppm (corr. to 0, 88, 564, 3120 mg/kg bw/day for males and 0, 104, 618, 2610 mg/kg bw/d for females) (nominal in diet) Exposure: Daily administration for 4 and 12 weeks Half of the animals sacrificed and	No **NOAEL** was found. **LOAEL:** 1200 ppm (88 mg/kg bw/day in males; 104 mg/kg bw/day in females). **1200 ppm** (88 mg/kg bw/day in males; 104 mg/kg bw/day in females) Males: No mortality. Body weight was ~4 % decreased at 4 weeks, and at 12 week, , body weight gain 11 % and affected during the study, and was ~4 % decreased at 4 and 8% at the 12 week time point. Absolute liver weight was not significantly affected, but relative liver weight was 24 % increased (after 12 weeks). Non-significant (9 %) decrease in absolute testis weights after 4 weeks, and a significant ~50 % decrease after 12 weeks. Decreased spermatogenesis (after 4 and 12 weeks), seen as testicular tubule atrophy (5/5 animals after 12 weeks), appearance of giant cells (3/5 animals at 4 weeks), interstitial oedema in the testis (4/5 animals at 12 weeks), spermatogenic arrest in testes (in 5/5 animals at 4 and 12 weeks) and hypospermia in the epididymis (in 5/5 animals at 4 and 12 weeks). Females: No mortality. Body weights were not significantly affected during the study, with 5% and 10% decreases were seen at 4 and 12 weeks, respectively. BWG was 14 and 26% decreased at the 2 time points, respectively Absolute and relative liver weights were not significantly affected, but relative liver weights were non-significantly	Takagi et al. 1994

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
biochemical examinations were conducted after 16 hrs of starvation. All animals were studied for histological changes. Reliability: Klimish score 2	examined after 4 weeks (as an intermediate study) and the other half after 12 weeks.	increased by ~14 % after 4 weeks and ~22 % increased after 12 weeks. In the haematological and biochemical findings a significant decrease of hemoglobin (HGB) was observed at week 12. After 4 weeks absolute and relative ovary weights were significantly decreased (~24 %), whereas the 9 % decrease in absolute ovary weights seen after 12 weeks was not statistically significant. No histopathological findings in uterus and ovary. **6000 ppm** (564 mg/kg bw in males; 618 mg/kg bw/day in females): Males: Increased mortality (survival rate 50% - reported as 45 % at 12 weeks, 1 death before week 4) probably due to haemorrhage, and statistically significant decreases in food intake, reduced body weight (~ 44 % after 4 and 12 weeks, with negative body weight gain at 4 weeks), increased relative liver weights after 4 and 12 weeks, thymus atrophy and bone marrow hypoplasia. In the haematological and biochemical findings significant decreases in the red blood cells (RBC) (at week 12), in haemoglobin (HGB) (at week 12), in cholinesterase (CHE) activity (at week 4), in phospholipids (PL) (at week 4), in total cholesterol (T-CHO) and in free cholesterol (F-CHO) (after 4 and 12 weeks) and a significant increase of γ-glutamyl transpeptidase (γ-GTP) (at week 12) were observed. Significant decreases in absolute testes weights (after 4 and 12 weeks) and decreases in relative testis weight (after 12 weeks), decrease of spermatogenesis (after 4 weeks and hereafter), testicular atrophy and interstitial oedema in the testis (at 12 weeks), at week 4 and 12, atrophy of the seminal vesicles and prostate. Note that due to the high mortality rate, the group size in regard to data generated at 12 weeks was two (n=2). Females: No effect on survival, significantly decreased body weight (27 % after 4 weeks and 37 % after 12 weeks with suppression of body weight gain), significant increases in absolute and relative liver weights (after 4 and 12 weeks). In the haematological and biochemical findings significant decreases in hemoglobin (HGB) (at week 12), in glucose (GLU) (at week 4) and in cholinesterase (CHE) activity (at week 4 and 12) were reported. Significant increases in phospholipids (PL) (at week 4 and 12), in total cholesterol (T-CHO) and in free cholesterol (F-CHO) (at week 4 and 12) and γ-glutamyl transpeptidase (γ-GTP) (at week 4 and 12) were observed. Decrease in absolute ovary weights, atrophy of the ovaries and uterus, histopathological changes in ovaries and uterus (after 4 and 12 weeks). **30000 ppm** (3120 mg/kg bw/day in, males; 2610 mg/kg bw/day in female): Males: increased mortality (survival rate 20 % at week 12, 2 deaths at	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		week 4), decreased food intake, weight loss compared to weight at study beginning: body weights markedly reduced (~ 50 % of controls after 4 weeks and ~33 % after 12 weeks), increased relative liver weights by ~ 89 % at 4 weeks, thymus atrophy and bone marrow hypoplasia, diarrhoea, biochemical changes in blood but not urine. In the haematological and biochemical findings was observed a significant decrease of haemoglobin (HGB) (at week 4), glucose (GLU) (at week 4), cholinesterase (CHE) activity (at week 4). Although not significant, a decrease of platelets (PLT) was noted at week 12. Significant increase of phospholipids (PL) (at week 4), and total cholesterol (T-CHO) and free cholesterol (F-CHO) were significantly elevated at week 4 and non-significantly at week 12 Decrease in absolute (by ~ 69 %, after 4 weeks) and relative (by ~ 83 %, after 4 weeks) testis weight, decrease of spermatogenesis (at 4 and 12 weeks), testicular atrophy and interstitial oedema in the testis (at 12 weeks), epididymis atrophy, hypospermia and atrophy of the seminal vesicles and prostate Note that due to the high mortality rate n=1 in regard to data generated at 12 weeks. Females: Increased mortality (survival rate 30%- reported to be 36 % at 12 weeks, one female died at 4 weeks), decreased food intake, loss of weight, so body weights were decreased 39% at week 4 and 44% at week 12), increased relative liver weights (by ~ 148 % after 4 weeks and ~ 185 % after 12 weeks) and absolute liver weights (by ~ 51 % after 4 weeks and ~ 59 % after 12 weeks). There was a decrease in absolute ovary weights (by ~ 56 % after 4 weeks and ~ 40 % after 12 weeks) and relative ovary weights (by ~ 30 % after 4 weeks and ~ 32 % after 12 weeks). Histological examination revealed bone marrow hypoplasia and thymus. Histopathological changes also included atrophy of the ovaries, and uterus,. None of the doses investigated affected weights of brain, heart, lung, kidney, spleen, adrenals, pituitary or thyroids.	
Study 3: Chronic oral toxicity study 18 months exposure rat (Wistar) male/female. n=30/sex/dose, groups of	Test substance: DBMC CAS no. 119-47-1, oral: feed 0, 100, 300, 1000 ppm	**Controls:** Survival was 95% in males and 90% in females. **100 ppm** (4.23 mg/kg bw/day in males; 5.1 mg/kg bw/day in females) Males: No adverse effects on body and liver weights or on testes weights. Females: Survival was 100%. No adverse effects on body and liver weights or on ovary weights	Takagi et al. 1994

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
5/sex/dose sacrificed at 6 and 12 months. Food consumption recorded monthly, and general condition were observed daily. Body and organ weight measurements at 6, 12 and 18 months (n=5, 5 and 10/sex/dose, respectively) Haematological and serum biochemical examinations at 6 and 12 months Histological findings at 6, 12 and 18 months. Reliability: Klimish score 2	(corresponding to 0, 4, 12.7, 42.3 mg/kg bw/day for males and 0, 5, 15.1, 54.2, mg/kg bw/d for females) Exposure: daily administration for 6, 12 and 18 months	**300 ppm** (12.7 mg/kg bw in males; 15.1 mg/kg bw/day in females) Males: Survival rate was decreased by 4%, from 95 to 91%. Body weights were not significantly affected at any time point during the study, but were non-significantly decreased by 6 % after 18 months. Absolute liver weights were not significantly increased at 6, 12 or 18 months, but relative liver weights showed a significant 9 % increase at 18 months. Relative and absolute testes weights, as well as testes and epididymis histopathology were not affected. Females: Survival was 95%. No other adverse effects were noted. **1000 ppm** (42.3 mg/kg bw/day in males; 54.2 mg/kg bw/day in females) Males: Survival rates were unaffected, but a significant 9 % decrease in body weight was observed at 18 months. Significantly increased absolute (15-20 %) and relative (22-27 %) liver weights were seen at all three time points. This dose caused very severe effects on the testes, i.e. a 58, 69 and 75% decrease in absolute testes weight at the three time points respectively. Relative testes weights were also significantly decreased throughout the study (58-73 %). At all three time points, severe testis tubules atrophy, spermatogenic arrest and epididymis hypospermia was seen in all investigated animals (5/5 at 6 and 12 months and 19/19 at 18 months). Females: Body weight at 18 months was ≈ 27% depressed, corresponding to a BWG decrease of 34%. Significantly increased relative, but not absolute liver weights were observed at all time points (20-34 %). No changes were noted regarding weight or histopathology of the ovaries.	
Study 4: Sub-acute toxicity study TG for 28-day repeated dose toxicity testing of chemicals (Japan), GLP rat (Crj: CD(SD)) male/female, n=6/sex/dose.	Test substance: DBMC CAS no:119-47-1 Oral, capsule doses of 0, 50, 200, 800 mg/kg bw/day Exposure: Daily administration for 28 days	**50 mg/kg bw/day** Males: Terminal body weights were not affected. A non-significant 14% increase in absolute and a significant 13 % increase in relative liver weights was seen. Other organ absolute or relative weights (brain, heart, thymus, spleen, kidney, adrenal and testes) were not affected, except for a significantly decreased (8 %) relative lung weight. No effects seen on other absolute or relative organs weights. Significant elevated total protein (~6 %) and haemoglobin were seen. Weight of testes not affected, but histological examination of testes showed degeneration of step 19 spermatids (in 3/6 animals, mild) but no other adverse effects. Females: Body weights and organs weights were unaffected. Haematology and blood chemistry were not significantly affected and	Ministry of Health and Welfare Japan (1996)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Note: due to the inclusion of a recovery group for the control and the 800 mg/kg groups, n=12/sex/dose in the urinary measurements for the control and the 800 mg/kg group. 19 blood chemistry and 16 haematology endpoints investigated. Absolute and relative weights of brain, heart, lung, thymus, liver, spleen, kidney, adrenal, ovary. Histopathology of liver performed. Reliability: Klimish score 2	A 14-day recovery group was also included (control and high dose animals)	neither were any of the examined organ weights. There was no effect on ovary weights. **200 mg/kg bw/day** Males: Terminal body weights were non-significantly increased (5%). Absolute and relative liver weights were significantly increased (by 25 and 19 %, respectively). Significant increase of total protein (~6 %), Prothrombin time (~52 %) and partial thromboplastin time (APTT) (~23 %) was seen. Non-significant changes in liver histology (mild, 1/6 animals). Significant effects on sperm retention (mild, 6/6 animals), degeneration of step 19 spermatids (mild/moderate, 6/6 animals) and vacuolation of Sertoli cells (mild, 6/6 animals) was reported. Females: Body weights were non-significantly decreased (6%). Significant increase absolute and relative liver weights (13 and 19 %, respectively) and mild centrilobular hypertrophy of the in liver (in 1/6 animals) were recorded. Significant increase of partial thromboplastin time (APTT) (by ~16 %) and albumin (by ~11 %). Significantly increased relative adrenal weights (16 %). There was no effect on ovary weights. **800 mg/kg bw/day** Males: Body weights non-statistically increased (4%). Significantly increases in absolute and relative liver weights (30 and 28 %, respectively) were reported. Significant increases in albumin (~9 %), total protein (~6 %), as well as in prothrombin time (~101 %), partial thromboplastin time (APTT) (~41 %), and platelets (14.8 %) and non-significant changes in liver histology (mild, 1/6 animals) were noted. Significant changes in sperm retention (moderate, 6/6), degeneration of step 19 spermatids (moderate, 6/6) and vacuolation of Sertoli cells (mild, 6/6 animals). Females: Body weights not affected. Significant increase in absolute and relative liver weights (30 %) and mild changes in liver histology (1/6 animals). Significant increase of prothrombin time (by ~62 %) and partial thromboplastin time (APTT) (by ~33 %), and a significant decrease in aspartate aminotransferase (GOT) (by ~16 %) and blood urea nitrogen (BUN) (by ~19 %). Albumin was non-significantly elevated by ~8 %. There was no effect on ovary weights. **800 mg/kg bw/day with recovery** Males: No difference in body weight was seen. Statistically increased relative liver weight (13 %, thus less pronounced than without recovery) but no histological effects in liver. Also a minor decrease	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		(~4 %) i absolute brain weight was observed, however no significant decrease when looking at the relative weight. Significantly elevated total protein (~6 %), while mean corpuscular volume (MCV) were decreased by ~3 % and γ-glutamyltransferase by ~67 %. These decreases were only seen in the recovery group. Haematology parameters where thus less affected than in the 800 mg/kg bw/day group without recovery. Histopathology of testes showed significant effects in all investigated parameters; vacuolation of Sertoli cells (mild, 5/ 6 animals), sperm retention (moderate, 5/6 animals), degeneration of step 19 spermatids (moderate, 5/6 animals), giant cell formation (mild/marked, 4/6 animals), nuclear vacuolation of spermatids (mild/moderate, 4/6 animals) and a decrease in germ cells (mild/marked 2/6 animals). The testicular effects did not disappear after two weeks without exposure, and certain endpoints (e.g. giant cell formation, nuclear vacuolation of spermatids and decreased number of germ cells) seemed even more severely affected in the recovery group than in the 800 mg/kg group. <u>Females</u>: Body weights were not different from controls. Significant increases in absolute (~ 13 %) and relative (~15 %) liver weight, and in absolute (~8 %) and relative (~9 %) kidney weight. Mild changes in liver histology (1/6 animals). Significant changes in 1 out of 16 haematology endpoints seen as an increase of eosinophils from 0 to 1.2±1.5 %. This increase was only seen in the recovery group. No effect on ovary weigth was recorded.	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Study 5: Sub-chronic toxicity study with Male rats (F344/DuCrj (Fischer) Male mice (Crj: CD(ICR) n=8/dose for both mice and rats At study termination, repr. organ weights were excised and weighted, examined histologically and testosterone levels in serum were determined. Reliability: Klimish score 2	Test substance: 4 biphenols, including DBMC CAS no:119-47-1 Oral: dietary exposure with daily administration for 2 months rat: 600 ppm (38.6-58.0 mg/kg/ bw/day) mouse: 2500 ppm (371-447 mg/kg bw/day) (mean of 414 mg/kg/day).	**Rat study** **40-60 mg/kg bw/day** The terminal body weights were non-significantly higher (7%) than in the controls, despite slightly lower mean food intake. Only reproductive organ weights were determined. Absolute testes and epididymides weights were non-significantly decreased by 2 % and 12 %, respectively. Significant decrease in relative testicular (9 %) and epididdymal (18 %) weights were seen and histopathological changes, including vacuolisation of Sertoli cells (in 8/8 animals), disappearance of basement membrane (8/8 animals), degeneration of spermatids (7/8 animals), exfoliation 7/8 animals), retention (8/8 animals) and broken tails of elongated spermatids (in 7/8 animals). Moreover, the daily sperm production (DSP) was significantly decreased in treated animals (~30 %). Serum testosterone levels were not significant changed. **Mouse study** **371-447 mg/kg bw/day** No difference with control group in body weight gain, terminal body weight, absolute or relative weight of liver or kidney. No effect on absolute or relative weights of testes or sex accessory organ was seen with DBMC. Significant histopathological changes in testes, including giant cell formation (6/8 animals) and sloughing of seminiferous tubules (4/8 animals), and non-significant haemorrhage in testis (1/8 animals), dilated lumen of vacuolated and multinucleated spermatocytes (3/8 animals) and Leidig cell vacuolization (2/8 animals) were noted. Daily sperm production was not assessed. Serum testosterone levels were not significant changed.	Takahashi et al. 2006
Study 6: Sub-chronic toxicity study rat (Wistar) male/female, n=10 Reliability: Klimish score 2	Test substance: DBMC CAS no not stated. 0,100, 330, 1000 or 3000 ppm in the diet (males: 0, 7.41, 24.91, 75.65, 281.64 mg/kg bw/d; females: 0, 9.66, 31.30, 113.16,	No mortality or clinical signs, no relevant changes in clinical chemistry, were noted in any of the doses. **100 ppm** (7.41 mg/kg bw/day, male; 9.66 mg/kg bw/day, female): no adverse effects. **330 ppm** (24.91 mg/kg bw/day, male; 31,3 mg/kg bw/day, female): no adverse effects. **1000 ppm** (75.65 mg/kg bw/day, male; 113.16 mg/kg bw/day, female): <u>Males:</u> Terminal body weight was non-significantly increased (2.6 %) and body weight gain was also higher (2.8%) than in controls. A non-	Bomhard et al. 1982

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	345.40 mg/kg bw/d) Exposure: daily administration for 13 weeks	significant increase in absolute liver weight (10 %) and a significant increase in relative liver weights (7 %) were seen. Very severe reduction in absolute (64 %) and relative testis weight (66%) was observed along with severe atrophy of the testes. Females: No significant effect on terminal body weight (7%) reported, and. body weight gain was decreased (≈ 6%) was seen. Absolute liver weight was not significantly increased (9 %) whereas relative liver weight was significantly increased (13 %). Histologically, no adverse effects on reproductive organs were noted. **3000 ppm** (281.6 mg/kg bw/day, male; 345.4 mg/kg bw/day, female): Males: Body weight and body weight gain were non-significantly decreased at study termination (6.6 and 8.9 %, respectively). A small non-significant increase in absolute liver weight and a significant increase in relative weight (3 and 7 % respectively) were seen. The changes in the testes were quite severe with and almost 60% decrease in relative and absolute testes weight and severe atrophy of the testes and epididymes in all 10 males. Females: A small but significant reduction in body weight (5%) and decrease in body weight gain (10%) was reported at sacrifice. Absolute and relative liver weights were significantly increased (24 and 31 % respectively). Atrophy of both uterus horns was observed in 4/10 females.	
Study 7: Sub-chronic toxicity study Male and female rats; Nelson strain albino rats, n=25/sex Note that not all parameters were examined for all animals: The number of animals varies from n=5-15/sex depending on the parameter assessed Reliability: Klimish score 2	Test substance: DBMC CAS no. not stated Oral: feed 0, 330, 1000, 3000 ppm, corresponding to 0, 25, 80.3, 241 mg/kg bw/day (males) and 0, 31.7, 92.2, 275 mg/kg bw/day (females) Exposure: daily administration for 90 days	**330 ppm** (males: 25 mg/kg bw/d; females: 31.1 mg/kg bw/d). No adverse effects seen in male or female rats. **1000 ppm** (males: 80.3 mg/kg bw/d; females: 92.2 mg/kg bw/d). Males: One male died, the possible relation to treatment unclarified. Mean body weight not affected. There was an increased mean liver weight, but no effect on the mean kidney weight. Atrophy of the testes (10/14 animals) was reported. Females: No adverse effects on body or organ weights (liver and kidney weights assessed) or at histology of any organs. There was no adverse histological finding in the ovaries. **3000 ppm** (males: 241 mg/kg bw/d; females: 275 mg/kg bw/d): Males: One male died, the possible relation to treatment unclarified. Food intake was decreased. Mean body weight was significantly lower (13%) than in the control group at study termination. Liver and kidney weights were increased. Atrophy of the testes (14/14 animals) were registred., Females: No mortality occurred. Food intake was decreased. The mean body weight was non-significantly depressed (7%). No adverse effects and organ weights (liver and kidney weights assessed) were	American Cyanamid Company 1965a

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		noted.No adverse histological findings of any organs were reported, The ovaries were not affected.	
Study 8: Sub-chronic toxicity study Dog (Beagle) N=2 per dose per sex. 2 separate feeding trials were conducted. Reliability: Klimish score 3	Test substance: DBMC CAS no:not stated Oral: dietary exposure with daily administration for 135 days in the first trial and 122 days in the second. First trial dietary levels of 330 ppm, 1000 ppm and 3000 ppm. Second study: 100 ppm and 200ppm were chosen.* *conversion to mg/kg bw/day not possible due to lack of information on daily feed consumption .	The reliability of this study is very limited. The study started out with a low group size of two animals per sex per dose, and since one male and one female dog died after 59 and 113 days of exposure to 3000 ppm , this further reducing group size in this dose. Due to the small group size no statistical evaluation of organ weights was performed, but the conclusion from study authors was that no adverse effects on organ weights were seen, whereas exposure levels above 330 ppm produced histopathological changes in the liver and pancreas. At dietary levels of 330 ppm and higher, also plasma alkaline phosphatase activity was significantly increased. No effect on food intake or body weight was seen at dietary level of 1000 ppm or less.	American Cyanamid Company 1965b

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

In males, the results from the reproductive/developmental toxicity screening study and the repeated dose toxicity studies in rodents presented above consistently show dose-related adverse effects on male sexual function and fertility following exposure to DBMC, including severely reduced testes and epididymal weights, testis tubules atrophy, spermatogenic arrest and changes in the sperm motility, viability and morphology. These effects have been reported in rat studies ranging from 28 day to 18 months exposure, occurring at dose levels from approximately 40 mg/kg bw/day. Similar effects have also been seen in a mouse study with 2 months of exposure.

The available information as presented in Annex II (WoE analysis) indicates that there is a dose-response and temporal concordance for the male reproductive toxicity being present from doses and duration where no other toxicity is present.

The adverse effects on male fertility after DBMC exposure were reported from dose levels of 40-88 mg/kg bw/day, whereas these doses entailed no to moderate general toxicity (reduction in body weights of 0-9% across the studies and relative liver weight increase from 0% up to 30% The observed effects in male reproductive parameters at these doses are not considered to be secondary non-specific consequences of other toxicity. The DS evaluates that moderate general toxicity effects to the body and liver weights reported in some studies at doses from around 200 mg/kg bw is not expected to yield specific effects to the testes and sperm. Thus, the effects at those doses are regarded relevant for classification. In one study of 4 and 12 weeks duration (study 2), doses larger than 500 mg/kg bw/day induced marked toxicity, such as mortality, severe body weight loss and large increases in liver weights were seen together with severe effects on testes and sperm,. These doses are used for classification on their own, but are included in the weight of evidence analysis (Annex II). In two other studies of 28 and 50 days of exposure, severe effects on testes were reported at 800 kg/kg bw without other marked toxicity.

A single subchronic toxicity study in Beagle dogs was available. In this study no adverse effects on testes histopathology were noted at any dose, although exposure levels above 330 ppm produced histopathological changes in the liver and pancreas. However, due to the very limited statistical power of the study (n=1-2/sex), and uncertainty on the dose levels in mg/kg bw/day, the study was not considered reliable for the overall evaluation.

Adverse effects on the female reproductive system were reported in 3 rodent studies, but not in 3 others. One study reported decreased ovary weights from 104 mg/kg bw in the 4 week trial, but not at 12 weeks at the same dose level, whilst 618 mg/kg bw/day caused ovary and uterus weight depression and histological changes of the two organs at both exposure times. In another study, 346 mg/kg bw/day over 13 weeks caused uterus horn atrophy. In the highest dose of the reproduction screening study (800 mg/kg bw/day), decreased number of corporae luteae and implantation scars were seen. There was no or moderate toxicity to body and liver weights and haematological parameter in these studies, except in the dose of 618 mg/kg bw/day, where the toxicity was pronounced. However, as the findings on female fertility are not found at similar doses and exposure time across the available studies do not permit to conclude on the possible effect of DBMC on female sexual function and fertility.

10.10.2.1 Weight of evidence analysis of adverse effects on sexual function and fertility

A Weight of Evidence (WoE) analysis of the findings on male reproductive toxicity (annex II to this document) has been performed using the "Weight of Evidence approach template" as available at https://echa.europa.eu/support/guidance-on-reach-and-clp-implementation/formats.

The analysis includes a summary of the assessment of the quality of the studies, performed according to the criteria defined within ECHA Guidance R.4 including assessment relevance, reliability and adequacy. Also, the WoE analysis provides an evaluation of the specific effects reported in the studies with DBMC on male reproductive functional parameters and on male reproductive organs with respect to incidence, severity, dose response and temporal concordance. Finally, an integration step is performed using additional elements (consistency, specificity and plausibility).

Study quality

The quality of the experimental evidence used has been analysed both at individual study level using the Klimisch scoring and integrated level using the WoE analysis presented in Annex II of this document. The details of the quality assessment are elaborated in Annex II.

Dose-response and temporal concordance

As elaborated in the WoE analysis in Annex II, an analysis of the relationship of the available evidence with respect to effects and their severity seen on sperm and testes, respectively, in relation to time (exposure periods) and doses of the registered substance shows high concordance, consistency and specificity: All the available studies in rodent species show the male reproductive system as the target of DBMC toxicity.

The effects are overall showing increasing incidence and severity with increasing doses and increasing exposure time, although some variability e.g. on sperm effects between different studies of the same duration can be attributed to expectable biological variation. The dose-response and temporal concordance analysis shows that the parameters related to the male functional fertility (sperm effects) are affected prior (at lower dose and following shorter exposure time) to those related to structural alterations in male reproductive organs (morphological effects on testes). The effects occur from doses without other systemic toxicity in the male rodent species and are regarded as non-secondary to such toxicity.

Mode of Action

A possible mode of action of DBMC, suggested by Takagi et al. (1994), is the molecular mechanism of uncoupling in mitochondria. Takagi et al. (1994) showed that DBMC, and a structurally similar anti-oxidant (2,2'-methylenebis (4-ethyl-6-tert- butylphenol); MBEBP or DEMC; CAS no 88-24-2) exert an uncoupling action in isolated liver mitochondria. The analogue has also caused atrophy in testicular tubules and decrease of spermatogenesis in subchronic toxicity studies in rodents.

Thus DBMC could inhibit the mitochondrial energy production in certain cells, resulting in a lack of ATP, which is necessary for cell division. Should this uncoupling in mitochondria be a dominant mode of action of DBMC *in vivo*, it could possibly explain why adverse effects occur in the testes at lower doses of DBMC that any other organs, as testes are organs with a very high level of cell division and consequently a high energy consumption. However, no experimental data are presently available to confirm this possible MoA of DBMC. Also, no data on the effects or mechanisms of action in humans are available.

Human relevance

There are no data available on the toxicokinetics for DBMC in animals or humans which would suggest species differences in toxicokinetics between animals and humans.

The available negative dog study is not considered to be reliable due its low quality, and there is thus no evidence that can overwrite the findings from multiple rodent repeated dose toxicity studies demonstrating severe effects on DBMC on the male reproductive system.

Therefore, the available evidence is considered sufficient to conclude relevance for humans of the effect of DBMC on reproduction.

The database on DBMC does not contain a generation study. A testing proposal from the lead registrant under REACH in order to fulfil the standard requirements for reproduction toxicity is currently under scrutiny by the Commission and ECHA. Although a full generation study is not available, the available evidence this is considered sufficient to conclude on classification of DBMC for reproductive toxicity.

Biological plausibility is high in terms of linking the demonstrated effects on testes and sperm to subsequent male infertility for humans: as stated in the OECD Guidance on mammalian reproductive toxicity testing and assessment (OECD, 2008): *"Histopathological changes is a more sensitive indicator of reproductive toxicity than are reduced fertility. Decreased fertility as revealed by effect on fertility index is a rather insensitive endpoint in rats. This may be explained by the rather high sperm reserve available in rats compared to humans."*

Due the fact that rats and mice have a tremendous excess of spermatozoa in their ejaculates, and as such sperm counts have to be reduced by as much as 90 % to affect fertility, a reduction in sperm count may not result in reduced fertility particularly in rodent studies. It is noted that sperm concentrations in man are highly variable and generally lower than in rodents. The distribution of sperm counts is currently such that many men have sperm concentrations near or below WHO reference values for fertility. Therefore, even a small decrease in sperm concentration across a population would be expected to shift the fertility potential of the group and move some men into the infertile or subfertile range. For this reason, a statistically significant change in sperm count in a rodent study is considered to be indicative of a potential effect on fertility in humans.

10.10.3 Comparison with the CLP criteria

Criteria for classificationas Repr. 1*: "Known or presumed human reproductive toxicant: Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility,[…] in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans."*

Classification of DBMC for adverse effects on sexual function and fertility in category 1 is considered appropriate based on clear evidence from the available data showing that the substance has the capacity to interfere with reproduction in rats.

Criteria for classification as Repr. 1A: *"Known human reproductive toxicant. The classification of a substance in this Category 1A is largely based on evidence from humans."*

Classification of DBMC for adverse effects on sexual function and fertility in category 1A is not appropriate as this category is used for known human reproductive toxicants. No human data specific for DBMC is available.

Criteria for classification as Repr. 1B: "*Presumed human reproductive toxicant. The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility […]in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."*

Classification of DBMC for adverse effects on sexual function and fertility in category 1B is considered appropriate.

The available data on reproductive effects of DBMC (one screening reproduction toxicity study and seven repeated dose toxicity studies rodents and one in dogs), a weight of evidence analysis (Annex II) of all available studies concluded that the quality of studies and reporting was overall good and allowed for conclusion of the toxicity to sexual function and fertility of the substance.

The data available on DBMC show clear evidence of adverse effects on sexual function and fertility from several studies in rodents as demonstrated in the WoE analysis (Annex II). The key results regarding adverse effects on sexual function and fertility are the following:

- Consistent reductions in absolute and relative weight of testes with effect levels beginning at 42 mg/kg/day when exposure was above 6 months (and at doses of 75-200 mg/kg/day for exposure duration was 1-2 months).
- Adverse histopathological effects in the testes, including giant cell formation and degradation of stem 19 spermatids at lower dose levels and testis tubules atrophy at higher dose levels.
- Constistently reduced sperm count and sperm motility and adverse effects on morphology parameters (Takagi et al 1994, MHWJ 1996a, 1999b, Takahashi et al 2006).

Dose-response relationships on the adverse effects on male fertility (testes morphology and semen quality) were clearly demonstrated, especially within studies and also appeared consistent across studies.

All these adverse effects on the male reproductive system were seen from doses where no, or in some cases minimal signs of other toxic effects (including small to moderate increases in liver weights, and alterations in a few blood parameters) were seen. The data on DBMC fulfil the requirement of the classification criteria that the effects seen should not be "*a secondary non-specific consequence of other toxic effects."*

The classification criteria further state (point 3.7.2.3.2):"… *If it is conclusively demonstrated that the toxicokinetic differences (between test animals species and humans) are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals should not be classified*."
However, as no toxicokinetic studies have been performed with DBMC (in either animals or humans), there is no data to indicate toxicokinetic differences for DBMC that would result in the chemical's hazardous properties not being expressed in humans. At present very little is known about the mechanism or mode of action leading to the observed adverse effects on the rodent testes. Uncoupling in the mitochondria has been suggested, leading to inhibition of the mitochondrial energy production in certain cells, resulting in a lack of ATP, which is necessary for cell division (Takagi et al 1994). This hypothesis has, however, not yet been substantiated by any experimental data *in vivo*. There are therefore no available data showing that the mode of action by which DBMC exerts its toxic effects on the rodent testes, would not be relevant for humans.

The classification criteria (point 3.7.5.2-3) also describe that: *"Results obtained from Screening Tests (e.g. OECD Guidelines 421[…]) can be used to justify classification, although it is recognised that the quality of this evidence is less reliable than that obtained through full studies. Adverse effects seen in short- or long-term repeated dose toxicity studies, which are judged likely to impair reproductive function and which occur in the absence of significant generalised toxicity, may be used as a basis for classification, e.g. histopathological changes in the gonads."*
As described above, the histopathological changes in the testes and effects on sperm are regarded as more reliable than e.g. fertility index for the evaluation of potential for effects on humans, due to the high sperm capacity of rodents. The consistent findings of histopathological changes in the testes and effects on sperm maturation, viability and motility from the above presented repeated dose toxicity studies concurring with the results of the screening reproduction toxicity study support the proposal of classification as toxic to reproduction in category 1.B.

The findings in females on effects of DBMC on sexual function and fertility in some studies are inconclusive, as they occur sporadically across the available studies. However, the findings may be regarded as supportive to the effects on male reproductive system.

Criteria for classification as Repr. 2:
Suspected human reproductive toxicant Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

Classification of DBMC for adverse effects on sexual function and fertility in category 2 is not appropriate as there is sufficient evidence to place the substance in category 1B. The quality of the evidence, as presented in a WoE approach (Annex II of this document), demonstrates reliability, adequacy and relevance of experimental evidence in animals. The analysis shows a consistent and specific severe effect for male reproductive toxicity which is sufficiently convincing to place the substance in Category 1.

Based on the above results and comparison with the classification criteria, it is concluded that DBMC fulfils the criteria for classification for reproductive toxicity in category 1B for effects on sexual function and fertility. The available data point at the testes and formation and function of sperm as the critical effects of DBMC. As there is only information on the oral route, and no mechanistic information to support or refute the possibility of effects by other exposure routes, it is proposed not to indicate a specific route of exposure.
The resulting proposal for classification is: ***Repr 1B; H360F: May damage fertility***

10.10.3.1 Specific concentration limits

The criteria for setting SCLs included in the Guidance on the Application of the CLP Criteria (version 5.0, July 2017) is based on intervals for ED_{10} values for the effect on which classification is based. The criteria include a low, medium and high potency group defined as shows in the table below:

Potency group	Boundaries
High potency group	ED_{10} value ≤ 4 mg/kg bw/day
Medium potency group	4 mg/kg bw/day < ED_{10} value < 400 mg/kg bw/day
Low potency group	ED_{10} value ≥ 400 mg/kg bw/day.

In the case of DBMC, classification is based both on testes and sperm effects. An estimation of ED_{10} from the available studies can be based on the incidences and severity of end-points on these parameters. The lowest effect levels (LOAELs) for both testes and sperm effects are around 40 mg/kg bw/day across the available studies on DBMC and NOAELs are demonstrated to be around 12.5 mg/kg bw/day.
The number of animals with moderate to severe effects on sperm and/or testes differs across studies depending on dose and exposure duration. At around 50 (40-88) mg/kg bw/day, the number of animals moderately to severely affected with respect to end-points supporting classification varies between e.g. a 20 % decreased sperm survivability ratio in the screening toxicity study (study 1) (ED_{20}), testicular tubule atrophy and spermatogenic arrest in 5/5 animals (ED_{100}) after both 4 and 12 weeks in a subchronic toxicity study (study 2) respectively whilst only mild effects on spermatids 3/6, with no effects on testes occurred in another 4 weeks study (study 4).
Whilst the establishment of a precise ED_{10} from this information is not readily possible, it appears that the ED_{10} would probably be between the NOAEL and the LOAEL of 40-88 mg/kg bw/day. The ED_{10} for DBMC would thus be expected to be lower than 40, but higher than 12.5 mg/kg bw/day, which would indicate that DBMC would be placed in medium potency group with respect of SCLs. Thus, ***no specific concentration limit is proposed for the end-point of reproductive toxicity, sexual function and fertility.***

10.10.4 Adverse effects on development

Information on developmental effects of DBMC can be retrieved from a developmental toxicity study in the rat and the reproduction / developmental toxicity screening test. Relevant findings from these two studies are thus included in table 10 below.

Table 10: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Developmental toxicity study/teratogenicity rat (Wistar), n=24-20-20-22(20).	oral: gavage 93.5, 187 or 375 mg/kg bw/day Exp: 7th to 17th day of pregnancy (daily) with sacrifice on	**93.5 mg/kg bw/day** No adverse effects on the dams or pups **187 mg/kg bw/day** Dams: Diarrhea, hair fluffing, suppression of body weight gain (approximately 6 % decrease in body weight on gestation day 20) and suppressed food consumption. No effect on mean number of corpora luteae was seen. Foetuses: no effects on number of implants or live foetuses, offspring	Tanaka et al. 1990

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	GD 20.	body weights or malformation incidence were observed. **375 mg/kg bw/day** Dams: Two dams died during the study. Diarrhoea, hair fluffing, decreased food consumption and suppression of body weight gain (appr. 16 % decrease in body weight on gestation day 20) were seen. No effect on mean number of corpora luteae or number of implants was seen. Foetuses: No. of live foetuses and foetal body weights were unaffected. However non-significant increases in the number of dead implants (69 compared to 26 in controls) and in dams with only dead implants (5 out of 20 compared to 1 out of 24 in controls) were seen, and therefore offspring from only 15 litters could be examined for malformations. No foetuses showed skeletal malformations and no significant differences were seen in no. of foetuses with variations. NOAEL maternal: 93.5 mg/kg bw/day NOAEL development:187 mg/kg bw/day	
Study 1: OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test), GLP rat (Crj: CD(SD)) male/female n=12/sex	Test substance: 2,2'-methylenebis(6-*tert*-betyl-*p*-cresol Screening oral: gavage 0, 12.5, 50, 200, 800 mg/kg bw and day (nominal) Exposure: male: 50-52 d, female: 40-48 d (from 14 days before mating to the day 3 of lactation) (daily)	***12.5 mg/kg bw/day:*** No adverse effects in females or offspring. ***50 mg/kg bw/day:*** Males: No adverse effects noted. Dams: No adverse effects were noted, including no decrease in body weight or body weight gain. No effects on ovary weights or any adverse effects on fertility were seen. Offspring: No significant effects reported. ***200 mg/kg bw/day****:* Males: no effects on body weights of food consumption at study termination. No records on general toxicity or on reproductive system at mating. Dams: Body weights were not affected during pre-mating period, whilst corrected body weights were slightly decreased (1%) at PND 0 and significantly decreased (by 7 %) at PND 4 compared to controls. Body weight gains (corrected) were non-significantly reduced compared to controls during the dosing period until delivery (5%). This effect became significant in the lactation period (11% at termination). Additionally, significantly lower food consumption was seen periodically during pre-mating, pregnancy and lactation. The fertility index (pregnant/ successfully mated) was 92%. Absolute and relative ovary weights were not significantly affected at necropsy on PND 4, neither were the number of corpora lutea and implantation scars. Offspring: The delivery index (no. of pups born /no. of implantation scars x 100) was however significantly decreased, indicating possible loss of foetuses during pregnancy. A decrease in number of live pups born and live pups on day 4 of lactation (~12 %) was seen. Both male and female offspring had higher birth weights than control pups, possibly due to the smaller litter size. Litter weights were unaffected at PND 0, but	Ministry of Health and Welfare Japan, MHWJ, (1999)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		significantly reduced at PND 4 (9%). ***800 mg/kg bw/day:*** Males: No effects on body weights at study termination. Dams: Corrected body weights were decreased at parturition (10%) and (9 %) at necropsy. Significant depression of corrected body weight gain during pregnancy (30%) and lactation periods (6,6%), and lower food consumption periodically during pre-mating, pregnancy and lactation.. The fertility index was 100%. Ovary weights were not affected. A significant 14 % decrease in the number of corpora luteae and a significant 8 % decrease in number of implantation scars were seen. Offspring: Delivery index was not significantly affected even though the number of pups born was significantly (9.6 %) decreased. One dam was unable to deliver pups, and 1 dam lost all pups during lactation. Offspring and litter weights at birth were unaffected, but at PD 4 ~10 % and ~16 % decreases in pup weights and litter weights, respectively, were recorded.	

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

In the prenatal developmental toxicty study in rats (Tanaka et al 1990) a dose of 93.5 mg/kg bw/day from GD7-17 caused no adverse effects in dams or offspring. Dams receiving a dose of 187 mg/kg bw/day showed slight signs of general toxicity (diarrhea, hair fluffing, suppression of BW gain (approximately 6% decrease in BW on GD20) and suppressed food consumption). No adverse effects on mean number of corpora luteae, implants and live foetuses, and no effects on offspring body weights and malformations were seen at that dose.

At the highest tested dose of 375 mg/kg bw/day, two dams died during the study. Other dams were affected by diarrhoea, hair fluffing, decreased food consumption and suppression of BW gain (appr. 16 % decrease in BW on GD20). At this dose also the mean number of corpora luteae, no. of implants and no. of live foetuses and fetal body weights were not affected. However, a non-significant increase in dead implants (69 compared to 26 in controls) and in dam with only dead implants (5/20 compared to 1/24 in controls) was seen, and therefore offspring from only 15 litters could be examined for malformations. No foetuses showed skeletal malformations and no significant difference were seen in the number of foetuses with variations.

In the reproduction/developmental toxicity screening study (MHWJ, 1999b), no effects on dams or offspring were reported up to 200 mg/kg bw/day. At 200 mg/kg bw/day, periodically lower food consumption was seen during pre-mating, pregnancy and lactation were seen in the dams. One percent reduction in body weight gain (corrected) was seen at parturition. Also significantly reduced corrected body weights (by 7 %) and reduced body weight gains at PND 4 (11%) were reported.

In the offspring treated with 200 mg/kg bw/day, significant decreases in delivery index, number of live pups at delivery and on day 4 of lactation (~12 %) were reported, whilst both male and female offspring had higher birth weights than control pups.

The dose of 800 mg/kg bw/day caused depression of body weight gain (30%) during the pregnancy and lactation periods (7%), and lower food consumption was seen periodically during pre-mating, pregnancy, and lactation. At necropsy body weight was 9 % decreased. Decreases in the numbers of corpora luteae (14 %) and implantation scars (8 %) were seen.

At this dose level, one dam was unable to deliver pups, and another dam lost all pups during lactation. A small but significant decrease in number of pups born was also reported. The birth weights of offspring were ~10 % decreased at this dose level.

Taken together, the results from the two available studies with DBMC investigating developmental toxicity, the prenatal developmental toxicity study (Tanaka et al 1990) and the screening reproduction/developmental study (MHWJ 1999) do not indicate any teratogenic effects of DBMC. Foetotoxicity was seen in two high dose groups (200 and 800 mg/kg/day) of the screening reproduction/developmental study. However, there was no clear dose-response of this effect on the developing foetus, and the effect it is not clear, whether the effects on the offspring was related to maternal toxicity. The non-significant decreased in number of live-born fetuses in the developmental toxicity at a dose of 375 mg/kg study and the reduced number of pups in the high dose groups in the screening study indicate a developmental toxic effect of DBMC. However, the effects were probably caused by maternal toxicity.

Overall, there are no indications of critical developmental effects of DBMC based on the available data.

10.10.2 Comparison with the CLP criteria

Criteria for classification as Repr. 1: *"Known or presumed human reproductive toxicant": Substances are classified in Category 1 for reproductive toxicity when they are known to have produced and adverse effect […] on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans."*

Classification of DBMC for adverse effects on development in category 1 is not considered as there is no strong presumption of the substances capacity to interfere with reproduction in humans, based on the available evidence in animals.

Criteria for classification as Repr. 2: "*Suspected human reproductive toxicant. Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on […] on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.*

Based on an analysis of the available data it is assessed that the observed decreases in number of live-born offspring at doses of 200 and 800 mg/kg/day in the reproduction sereening test and at 375 mg/kg bw/day in the developmental study may be related to parental toxicity, e.g.. mortality of dams, decreased food consumption and suppression of body weight gain, and possible effects on reproductive function of the male parents.The lack of toxicity in the developmental study. As only one species was investigated, the available data on a possible developmental effect of DBMC is insufficient to conclude that DBMC exerts an adverse, specific and non-secondary effect on development. Therefore, classification for developmental toxicity of DBMC is not considered to be warranted.

10.10.3 Adverse effects on or via lactation

No data available.

10.10.4 Overall conclusion on classification and labelling for reproductive toxicity

Based on the evaluation of the available information, it is concluded that classification as toxic to reproduction in category 1B for sexual function and fertility, without attribution of specific concentration limits, is warranted for DBMC, with no indication of critical effect or route of exposure.

The resulting proposal is therefore: Repr 1B; H360F: May damage fertility.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Effects on sexual function and fertility

The dossier submitter (DS) included in the CLH report one reproductive/developmental toxicity screening study conducted according to OECD TG 421 and GLP in rats, and seven repeated dose toxicity studies in rodents not performed according to OECD Test Guidelines, and with only one performed according to GLP. In the repeated dose toxicity studies (six studies in rats, one study in mice and one study in dogs) the exposure ranged from 28 days to 18 months and male reproductive endpoints including testes histopathology and/or sperm parameters were assessed.

The reproductive/developmental toxicity screening study in rats and the repeated dose toxicity studies in rats and mice consistently showed dose-related adverse effects on male sexual function and fertility following exposure to DBMC. These included severely reduced testes and epididymis weights, testis tubules atrophy, spermatogenic arrest and changes in sperm motility, viability and morphology. These effects were reported in rat studies ranging from 28 day to 18 months exposure at dose levels from approx. 40 mg/kg bw/d. Similar effects were also reported in the mouse study following a 2-month exposure to one dose of DBMC (mean dose of 414 mg/kg bw/d).

The adverse effects in rats on male sexual function and fertility following DBMC exposure were reported from 40-88 mg/kg bw/d. At these dose levels no to moderate general toxicity (reduction in body weights of 0-9% across the studies and relative liver weight increases of 0% to 30%) were reported. The effects observed in male reproductive parameters at these doses were considered by the DS not to be secondary, non-specific consequences of other toxicity. Therefore, the effects were regarded relevant for classification.

A single sub-chronic toxicity study (122-135 days) in Beagle dogs was available (study No. 8 in the Table below). In this study no adverse effects on testis histopathology was noted at any dose, although exposure levels above 330 ppm produced histopathological changes in the liver and pancreas. However, due to the very limited statistical power of the study (n=1 to 2/sex), and uncertainties related to the dose levels used in the study in mg/kg bw/d (the information on daily feed consumption was lacking), the study was not considered reliable by the DS for the overall evaluation.

In the female reproductive system, adverse effects were reported in 3 repeated dose rodent toxicity studies, but not in 3 other repeated dose toxicity studies. In the studies showing adverse effects on the female reproductive system, no or moderate other toxicity was evident (decreased body weight and increased liver weights as well as changes in haematological parameters) except in one study at 618 mg/kg bw/d (study No. 2), where the toxicity was more pronounced. However, as the findings on the female reproductive system were not found at similar doses and exposure durations across the available studies, the DS considered that it was not possible to conclude on whether there was an effect of DBMC on

female sexual function and fertility.

Weight of Evidence analysis

In Annex II to the CLH report the DS included a weight of evidence (WoE) analysis of the effects reported on male reproductive functional parameters and on male reproductive organs (incidence, severity, dose response and temporal concordance). The WoE analysis included the following:*Dose-response and temporal concordance:* All the available studies in rats and mice showed that the male reproductive system was the target of DBMC toxicity. The effects showed overall an increasing incidence and severity of effects with increasing doses and exposure time in rats, although some variability e.g. on sperm effects between different studies of the same duration could be attributed to biological variation. The effects on the male reproductive organs were reported at doses without general systemic toxicity in rats and mice and were therefore not regarded as secondary to general toxicity.

Mode of Action (MoA): A possible MoA of DBMC, as suggested by Takagi *et al.* (1994), was a molecular mechanism of uncoupling in mitochondria. Should the uncoupling in mitochondria be a dominant MoA of DBMC *in vivo*, it could possibly explain why adverse effects occurred in the testes at lower doses of DBMC that in any other organs, since testes have a very high level of cell division and consequently a high energy consumption. However, no experimental data are presently available to confirm this possible MoA for DBMC. In any case, the DS concluded that there was no mechanistic data that would suggest that the MoA was not relevant to humans.

Human relevance: There were no data available on the toxicokinetics of DBMC in animals or humans which would suggest species differences in toxicokinetics. While the negative dog study was not considered reliable due to its low quality, the findings from several rodent repeated dose toxicity studies showed severe effects of DBMC on the male reproductive system. Therefore, the evidence was considered by the DS to be sufficient to conclude on the relevance for humans of the effects of DBMC on male reproduction. The database on DBMC does not contain a generation study. However, biological plausibility was considered to be high in terms of linking the demonstrated effects on testes and sperm to subsequent male infertility for humans: as stated in the OECD Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment (OECD, 2008): "*Histopathological changes is a more sensitive indicator of reproductive toxicity than are reduced fertility. Decreased fertility as revealed by effect on fertility index is a rather insensitive endpoint in rats. This may be explained by the rather high sperm reserve available in rats compared to humans*." Therefore, a statistically significant change in sperm count in a rodent study was considered to be indicative of a potential effect on fertility in humans.

Comparison with the CLP criteria

The DS proposal to classify DBMC as Repr. 1B; H360F was based on clear evidence of adverse effects on sexual function and fertility from several studies in rodents. The findings on sexual function and fertility in females in some of these studies were inconclusive, as they occurred sporadically across the available studies. However, according to the DS these findings may be regarded as supportive to the effects on the male reproductive system.

Setting of Specific Concentration Limits (SCL)

The classification of DBMC for effects on sexual function and fertility proposed by the DS is based on effects on testes and on sperm. The DS estimated an ED_{10} from the available

studies based on the incidences and severity of these effects. The lowest-observed-adverse-effect levels (LOAELs) for both testes and sperm effects were around 40 mg/kg bw/d across the available studies on DBMC, while the no-observed-adverse-effect levels (NOAELs) were demonstrated to be around 12.5 mg/kg bw/d. The number of animals with moderate to severe effects on sperm and/or testes was different across studies depending on dose and exposure duration and varied between e.g. 20% (study 1) and 100% (study 2) depending on dose and exposure duration. Therefore, the establishment of a precise ED_{10} from the available information was considered not readily achievable by the DS. However, the DS considered it probable that the ED_{10} would be lower than 40 mg/kg bw/d, but higher than 12.5 mg/kg bw/d, which would indicate that DBMC would be placed in the medium potency group (4 mg/kg bw/d < ED_{10} > 400 mg/kg bw/d) with the use of the generic concentration limit (GCL). Therefore, no specific concentration limit was proposed by the DS for sexual function and fertility.

Developmental toxicity

The DS included two studies in rats: a developmental toxicity study (no OECD TG or GLP) and a reproduction/developmental toxicity screening study (OECD TG 421 and GLP).

In the prenatal developmental toxicity study in Wistar rats (Tanaka *et al*., 1990) female rats were exposed on GD 7-17 to 0, 93.5, 187.0 or 375.0 mg/kg bw/d of DBMC by gavage. In the reproduction/developmental toxicity screening study, CD (SD) rats were exposed to 0, 12.5, 50, 200 and 800 mg/kg bw/d of DBMC by gavage (females from 14 days before mating to lactation day 3).

In summary, the results from these two studies did not indicate any teratogenic effects of DBMC. In the screening reproduction/developmental study, foetal -toxicity was seen in the two high dose groups (200 and 800 mg/kg/day) with a reduced number of live foetuses. However, this effect was observed without a clear dose-response relationship, and it was not clear whether the effects on the offspring were related to maternal toxicity. In the developmental toxicity study a non-significant decrease in the number of liveborn foetuses at 375 mg/kg bw/d was reported. The effects on foetal viability in the two studies were considered to indicate a developmental toxic effect of DBMC. However, the DS considered that these effects were probably caused by maternal toxicity.

Overall, the DS concluded that there are no indications of critical developmental effects of DBMC based on the available data, and no classification for developmental toxicity was proposed.

Comments received during public consultation

Comments were received from four Member States Competent Authorities (MSCAs).

Comments related to classification of DBMC for effects on sexual function and fertility were all in support for a classification as Repr. 1B; H360F based on the clear adverse effects seen on male reproductive organs as well as sperm parameters.

One MSCA commented on the developmental toxicity section and agreed with the DS that no classification for developmental toxicity was warranted based on absence of adequate data. In the developmental study (Tanaka 1990), the adverse effects on development were considered to occur only in the presence of marked maternal toxicity. In the screening reproductive toxicity study, a small increase in foetal mortality was observed in the mid dose

in the absence of a dose-response relationship (no effect at the high dose), but it was acknowledged that this study was performed with a limited number of animals per dose group, providing a low statistical power.

Assessment and comparison with the classification criteria

Effects on sexual function and fertility

For the assessment of sexual function and fertility, the DS included one reproductive/developmental toxicity screening study conducted according to OECD TG 421 and GLP in rats and seven repeated dose toxicity studies (not conducted according to OECD TG). In the repeated dose toxicity studies male reproductive endpoints including testes histopathology and/or sperm parameters were assessed in six studies in rats (three different strains), one study in mice and one study in dogs. The exposure duration in the repeated dose toxicity studies ranged from 28 days to 18 months. In the text that follows, the studies are referred to by the numbers indicated in the table below.

Study No.	Method/Guideline	Klimisch	Reference
1	Reproduction Developmental Toxicity Screening Test in rats, OECD TG 421, GLP: oral diet; 0, 1200, 6000, 30.000 ppm (m: 0, 88, 564, 3120 mg/kg bw/d; f: 0, 104, 618, 2610 mg/kg bw/d)	1	Ministry of Health and Welfare Japan (1999b)
2	Sub-chronic oral toxicity study, 4-12 weeks in rats, no information on TG/GLP: oral diet; 0, 100, 300, 1000 ppm (m: 0, 88, 564, 3120 mg/kg bw/d; f: 0, 104, 618, 2610 mg/kg bw/d)	2	Takagi *et al*. 1994
3	Chronic oral toxicity study, 18 months in rats, no information on TG/GLP: oral diet; 0, 100, 300, 1000 ppm (m: 0, 4, 12.7, 42.3 mg/kg bw/d; f: 0, 5, 15.1, 54.2 mg/kg bw/d; 6 , 12, 18 months)	2	Takagi *et al*. 1994
4	Sub-acute toxicity study, 28 days in rats, TG: 28-day repeated dose toxicity testing of chemicals (Japan), GLP: oral capsule; 0, 50, 200, 800 mg/kg bw/d	2	Ministry of Health and Welfare Japan (1999b)
5	Sub-chronic toxicity study, 2 months, with male rats and mice, no information on TG/GLP: oral diet; rat: 600 ppm (38.6-58.0 mg/kg bw/d); mouse: 2500 ppm (371-447 mg/kg bw/d)	2	Takahashi *et al*. 2006
6	Sub-chronic toxicity study, 13 weeks in rats, no information on TG/GLP: oral diet; 0, 100, 330, 1000 or 3000 ppm (m: 0, 7.41, 24.91, 75.65, 281.64 mg/kg bw/d; f: 0, 9.66, 31.30, 113.16, 345.40 mg/kg bw/d)	2	Bomhard *et al*. 1982
7	Sub-chronic toxicity study, 90 days in rats; no information on TG/GLP: oral diet; 0, 330, 1000, 3000 ppm (m: 0, 25, 80.3, 241 mg/kg bw/d; f: 0, 31.7, 92.2, 275 mg/kg bw/d)	2	American Cyanamid Company 1965a
8	Sub-chronic toxicity study, 135 and 122 days in dogs, no information on TG/GLP: diet; 1st trial: 330, 1000, 3000 ppm; 2nd trial: 100, 200 ppm	3	American Cyanamid Company 1965b

Reproduction/developmental toxicity screening study

The main study (No. 1) for the assessment adverse effects on sexual function and fertility was a reproduction/developmental toxicity screening test according to OECD TG 421 in rats exposed by gavage to DBMC. In this study, CD (SD) rats were exposed to 0, 12.5, 50, 200

and 800 mg/kg bw/d DBMC from 14 days before mating to lactation day 3.

General toxicity

Females: At 200 mg/kg bw/d a significantly reduced corrected body weight (7 %) and reduced body weight gain at lactation day 4 (11%) compared to controls were reported. In addition, significantly lower food consumption was seen periodically during pre-mating, pregnancy and lactation. At 800 mg/kg bw/d, body weight was not affected during premating. During pregnancy the maternal body weight was decreased (6%) as well as the corrected body weight (8%) compared to controls at pregnancy day 21. At necropsy, body weight was decreased by 9% as compared to controls. The body weight gain was depressed (29%) from study start until termination compared to control animals. Food consumption was periodically lower during premating, pregnancy and lactation, and occasional cases of loose stools and salivation were reported. However, no effects were seen in 9/12 animals.

Males: In the high dose group a transient decrease in food consumption (one day in the beginning of the dosing period) was reported, but there were no effects on body weight.

Effects on sexual function and fertility

Females: At the top dose, there were no effects on ovary weights, but a statistically significant (14%) decrease in the number of corpora lutea and a statistically significant (8%) decrease in the number of implantation scars were reported.

Males: Statistically significant effects were reported on the weight and histopathology of the testes and epididymis from 200 mg/kg bw/d and on sperm quality from 50 mg/kg bw/d in the absence of general toxicity (tables below). It should be noted that in the 200 and 800 mg/kg bw/d dose groups a reduction in the number of live pups born was reported, reaching statistical significance only at 200 mg/kg bw/d, however, without a dose-response relationship (for further details see the section on developmental toxicity).

Table: *Effects in rats on testes and epididymis weight*

Dose (mg/kg bw/d)	**0**	**12.5**	**50**	**200**	**800**
# animals	12	12	12	12	12
Testes (g)	3.55±0.33	3.60±0.32	3.56±0.30	2.98±0.77*	1.74±0.26**
Epididymis (g)	1.26±0.14	1.34±0.12	1.20±0.11	1.11±0.13*	0.92±0.10**

*p<0.05, **p<0.01

Table: *Histopathological effects in rats reported in testes and epididymis*

Dose (mg/kg bw/d)	**0**		**12.5**		**50**		**200**		**800**	
# animals	12		12		12		12		12	
Incidence and grade	#	±,+,2+,3+	#	±,+,2+,3+	#	±,+,2+,3+	#	±,+,2+,3+	#	±, +, 2+,3+
Testis										
Atrophy seminiferous	0		0		0		6	1, 2, 2,1 **	12	0,1,10,1 **##
Tubules degeneration	0		0		0		1	1, 0, 0, 0	0	
Decreased sperm	0		0		0		1	1, 0, 0, 0	0	
Giant cell formation	0		0		2	2, 0, 0, 0	2	2, 0, 0, 0	0	
Epididymis										
Decreased sperm	0		0		0		9	2, 2, 1, 4**	12	0,0,0,12 **##

**p<0.01, ## significantly different by dose response test p<0.01

Table: *Effects on sperm parameters in rats*

Dose (mg/kg bw/d)	0	12.5	50	200	800
# animals	12	12	12	12	12
Motility ratio (%)	72.0 ± 9.7	74.9 ± 7.8	60.4 ± 10.3**	14.5 ± 21.8**	0.0 ± 0.0**
Abnormal sperm ratio (%)	1.5 ± 3.6	0.5 ± 0.5	8.1 ± 6.3**	56.3 ± 29.0**[b]	91.7[a]
Viability (%)	98.6 ±2.0	99.6 ± 0.5	89.2 ±11.5**	71.7 ± 9.3**[c]	-
Survivability (%)	83.3 ± 6.9	86.4 ± 3.3	66.0 ± 17.8**	39.0 ± 15.2**[c]	-
# sperm left epididymis (x10^6)	207.4±60.2	222.4±49.3	128.0 ± 39.9**	60.7 ± 29.2**	30.3±16.0**
# sperm/g left epididymis (x10^6)	707.8±153.0	704.8±156.6	503.3± 159.4**	238.9± 102.4**	138.9±83.3**

** p<0.01, [a]: one male rat, [b]: 7 male rats, [c]: 4 male rats

To further assess the effects on male reproductive organs several repeated dose toxicity studies with durations from 28 days to 18 months were included by the DS in the CLH report.

Repeated dose toxicity studies in rodents, 6 rat studies and one mice study

Repeated dose toxicity study in Wistar rats (study No. 2)

The animals were exposed to DBMC in the diet at 0, 88, 564, 3120 mg/kg bw/d (males) and 0, 104, 618 and 2610 mg/kg bw/d (females) for 4 weeks (5/sex/dose) or for 12 weeks (5/sex/dose).

Results

Mortality was reported in the high dose group in males and females following 4 weeks of exposure and in the control, mid- and/or high dose group following 12 weeks. Body weight decreased from the mid-dose in males and females. The relative organ weight of the liver was increased from the mid-dose group in males and females. The relative weight of the testes was decreased in the high dose group following 4 weeks of exposure and from the mid-dose group after 12 weeks. For females, the relative ovary weight was decreased in the high-dose group following 4 and 12 weeks of exposure. Results on organ and body weights are presented below.

Table: *Body and organ weights in male and female rats*

Weeks	Dose (mg/kg bw/d)	Body weight (g)	# rats	Absolute liver weight(g)	Absolute testis/ovary weight (g/mg)	Relative liver weight (g %)	Relative testis/ovary weight (g %/mg %)
4 (M)	0	232±6	5	7.39±0.32	2.89±0.17	3.19±0.12	1.25±0.09
	88	222±12	5	8.34±0.40	2.62±0.16	3.76±0.11	1.18±0.09
	564	129±16**	5	6.91±0.97	1.26±0.32**	5.34±0.30*	0.99±0.31
	3120	111±6**	4	6.75±1.69	0.90±0.25**	6.03±1.17**	0.81±0.21*
12 (M)	0	314±18	4	8.12±0.57	2.99±0.10	2.58±0.05	0.95±0.06
	88	300±14	5	9.61±0.67	1.45±0.11**	3.20±0.10**	0.48±0.03**
	564	178±29**	2	7.95±0.72	0.84±0.10**	4.49±0.31**	0.47±0.02**
	3120	110	1	6.86	0.78	6.23	0.71
4 (F)	0	162±8	5	4.65±0.37	83±7	2.88±0.12	52±4
	104	154±3	5	5.08±0.43	62±6*	3.29±0.33	40±4*
	618	117±16**	5	6.50±1.23*	57±11**	5.54±0.46**	49±7
	2610	99±12**	4	7.03±1.08**	36±9**	7.13±0.72**	36±5**
12 (F)	0	188±12	5	4.40±0.19	66±11	2.34±0.16	37±5
	104	170±9	5	4.85±0.29	60±2	2.86±0.12	35±2
	618	118±29**	5	6.21±1.56*	34±11**	5.29±0.54**	29±4
	2610	105±5**	2	7.01±0.77*	26±3**	6.71±1.08*	25±1**

*p<0.05, **p<0.01

Histological findings were reported in the thymus and bone marrow in males and females and in the testes and ovary as shown in the table below.

Table: *Histological findings in male and female rats*

Males								
Findings	**4 weeks (mg/kg bw/d)**				**12 weeks (mg(kg bw/d)**			
	0	**88**	**564**	**3120**	**0**	**88**	**564**	**3120**
#male rats	5	5	5	4	4	5	2	1
Thymus atrophy								
±	0	0	4	0	0	0	2	0
+	0	0	0	4	0	0	0	1
Bone marrow hyperplasia								
±	0	0	2	2	0	0	1	0
+	0	0	3	0	0	0	0	0
++	0	0	0	1	0	0	0	1
Testes tubules atrophy								
±	0	0	4	2	0	0	0	0
+	0	0	1	2	0	0	0	1
++	0	0	0	0	0	0	1	0
+++	0	0	0	0	0	5	1	0
Giant cell appearance								
±	0	2	0	0	0	0	0	0
+	0	1	2	0	0	0	1	0
++	0	0	2	2	0	0	0	0
+++	0	0	1	2	0	0	0	0
Epididymis atrophy								
±	0	0	0	1	0	0	0	0
+	0	0	0	2	0	0	0	1
Hypospermia								
+	0	2	0	0	0	0	0	0
++	0	3	0	0	0	0	0	0
+++	0	0	5	4	0	5	2	1
Seminal vesicle atrophy								
±	0	0	0	1	0	0	1	0
+	0	0	2	2	0	0	0	1
Prostate Atrophy								
±	0	0	3	0	0	0	1	1
+	0	0	2	4	0	0	0	0
	Females							
	4 weeks (mg/kg bw/d)				**12 weeks (mg/kg bw/d)**			
	0	**104**	**618**	**2610**	**0**	**104**	**618**	**2610**
# female rats	5	5	5	4	5	5	5	2
Thymus atrophy								
±	0	0	1	1	0	0	3	0
+	0	0	0	1	0	0	0	2
Bone marrow hyperplasia								
±	0	0	3	2	0	0	2	1
+	0	0	0	0	0	0	1	0
++	0	0	0	0	0	0	0	0
Ovary atrophy								
±	0	0	3	3	0	0	3	2
Uterus atrophy								
±	0	0	2	4	0	0	2	2

Repeated dose toxicity study in Wistar rats (study No. 3)

The animals were exposed to DBMC in the diet at 0, 4, 12.7 and 42.3 mg/kg bw/d (males) and 0, 5, 15.1 and 54.2 mg/kg bw/d (females) for 6 (5/sex/group), 12 (5/sex/group) or 18

months (20/sex/group). In the control group the survival was 95% in males and 90% in females.

Results

Males: In the low dose group no effects were reported. In the mid-dose group, the survival rate was decreased by 4%, from 95 to 91%. The relative liver weight was significantly increased (9%) at 18 months. In the high dose group, a significant decrease (9%) in the body weight was reported at 18 months. Significantly increased relative liver weights (22-27%) were reported at all three time points. In the testes severe effects included a significantly decrease in the relative testis weights throughout the study (58-73%) at all three time points. Further, severe testis tubules atrophy, spermatogenic arrest and epididymis hypospermia was seen in all animals (5/5 at 6 and 12 months and 19/19 at 18 months).

Females**:** In the low- and mid-dose groups no effects were reported on body weight or on ovary weight. In the mid-dose the survival rate was 95%. In the high-dose group the body weight at 18 months was decreased (27%), corresponding to a body weight gain decrease of 34%. Significantly increased relative liver weights were observed at all time points (20-34%). No changes were noted regarding weight or histopathology of the ovaries.

Repeated dose toxicity study in CD(SD) rats (study No. 4)

The animals were exposed to 0, 50, 200 and 800 mg/kg bw/d DBMC by gavage for 28 days followed by 14 days recovery period (control and high dose animals) (6/sex/group, but 12/sex/high dose- and recovery group).

Results

Males: In the low dose group a significant (13%) increase in relative liver weights was reported. Other organ weights were not affected except for a significantly decreased (8%) in relative lung weight. The weight of the testes was not affected, but histological examination showed degeneration of step 19 spermatids (described as mild in 3/6 animals). In the mid-dose group, the absolute and relative liver weights were significantly increased (25% and 19%, respectively). In the testes a significant effect on sperm retention (mild, 6/6 animals), degeneration of step 19 spermatids (mild/moderate, 6/6 animals) and vacuolation of Sertoli cells (mild, 6/6 animals) was reported. In the high dose group, a significantly increase in absolute and relative liver weights (30 and 28%, respectively) were reported. In the testes significant changes in sperm retention (moderate, 6/6), degeneration of step 19 spermatids (moderate, 6/6) and vacuolation of Sertoli cells (mild, 6/6 animals) was reported.

Females: there were no effects on ovary weights in any dose groups tested. In the mid- and high dose groups, significant increases in absolute and relative liver weights (mid-dose: 13 and 19 %, respectively and high dose: 30%) as well as mild changes in liver histology (1/6 animals) were reported.

Recovery group Males: Statistically significantly increased relative liver weight were observed (13%, thus less pronounced than without recovery), but no histological

effects in the liver were reported. In the testes, histopathology showed significant effects in all investigated parameters, including vacuolation of Sertoli cells (mild, 5/6 animals), sperm retention (moderate, 5/6 animals), degeneration of step 19 spermatids (moderate, 5/6 animals), giant cell formation (mild/marked, 4/6 animals), nuclear vacuolation of spermatids (mild/moderate, 4/6 animals) and a decrease in germ cells (mild/marked 2/6 animals). The results showed that the effects in the testes did not disappear after two weeks recovery, and some of the parameters (e.g. giant cell formation, nuclear vacuolation of spermatids and decreased number of germ cells) were even more severely affected in the recovery group than in the 800 mg/kg bw/d dose group without recovery.

Recovery group Females: Significant increases were seen in absolute (approx. 13%) and relative (approx. 15%) liver weight, and in absolute (approx. 8%) and relative (approx. 9%) kidney weight. Further, mild changes in liver histology (1/6 animals) was reported. No effects on the ovary were reported.

Repeated dose toxicity study in male F344/DuCrj Fisher rats and male Crj:CD(ICR) mice (study No. 5)

The animals (8/sex/group) were exposed to a single dose of DBMC in the diet for 2 months, 600 ppm in rats (38.6 – 58.0 mg/kg bw/d) and 2500 ppm in mice (371-447 mg/kg bw/d).

Results in rats

No significant general toxicity was reported. In the testes a significant decrease in relative testicular (9%) and epididymis (18%) weights were reported. Histopathological examinations reported vacuolisation of Sertoli cells (8/8 animals), disappearance of basement membrane (8/8 animals), degeneration of spermatids (7/8 animals), exfoliation (7/8 animals), retention (8/8 animals) and broken tails of elongated spermatids (7/8 animals). Moreover, the daily sperm production (DSP) was significantly decreased in exposed rats by approx. 30%. Serum testosterone levels were not significant changed.

Results in mice

No significant general toxicity was reported. In the testes or sex accessory organs there were no changes in absolute or relative weights. Histopathological examinations reported significant changes in testes, including giant cell formation (6/8 animals), sloughing of seminiferous tubules (4/8 animals), dilated lumen of vacuolated and multinucleated spermatocytes (3/8 animals) and Leydig cell vacuolisation (2/8 animals). Daily sperm production was not assessed. Serum testosterone levels were not significant changed.

Repeated dose toxicity study in Wistar rats (study No. 6)

The animals were exposed to 0, 100, 330, 1000 and 3000 ppm DBMC in the diet for 13 weeks, corresponding, respectively, to approx. 0, 7.5, 25, 75 and 282 mg/kg bw/d (males) and 0, 10, 31, 113 and 345 mg/kg bw/d (females) (10/sex/group).

Results

In males and females no mortality or clinical signs, as well as no relevant changes in clinical chemistry, were reported in any of the doses tested. Further, no adverse effects were reported at the two lowest doses (100 and 330 ppm).

Males**:** At 1000 and 3000ppm, a significant increase in the relative liver weights (7%) were reported. In the testes a severe reduction in relative testis weight (approx. 60%) was observed along with a dose-related increase in severe testis atrophy. Further, in the high dose group severe atrophy in the testes and epididymis in all 10 animals was reported.

Females: At 1000 ppm the only effect reported was a significantly increase (13%) in the relative liver weight. At 3000 ppm a small but significant reduction in body weight (5%) and body weight gain (10%) was reported at sacrifice. The relative liver weights were significantly increased (31%). Further, atrophy of both uterus horns was observed in 4/10 females.

Repeated dose toxicity study in Nelson albino rats (study No. 7)

The animals were exposed to DBMC in the diet for 13 weeks to 0, 330, 1000 and 3000 ppm DBMC corresponding, respectively, to approx. 0, 25.0, 80.3 and 241.0 mg/kg bw/d (males) and 0, 32.0, 92.0 and 275.0 mg/kg bw/d (females) (5-15/sex/group).

Results

At the low dose, no adverse effects in male and female rats were recorded.

Males**:** At the mid- and high dose, one male died in each dose group but the relationship to treatment was unclear. There was an increase in the mean liver weight in both dose groups. In the high dose group, the food intake was decreased, and the mean body weight was significantly lower compared to the control group (13%) at study termination. The kidney weight was increased. In the testes, a dose-depended increase in atrophy was found in 10/14 rats at the mid-dose and 14/14 rats in the high dose group.

Females**:** No adverse effects were reported in the mid- and high dose groups.

Repeated dose study in dogs

Repeated dose toxicity study in Beagles (study No. 8)

The dogs were exposed to 0, 330, 1000 and 3000 ppm DBMC in the diet for 135 days in the first trial, and for 122 days to 100 and 200 ppm in the second trial. Conversion to mg/kg bw/d was not possible due to lack of information on daily food consumption (2/sex/group). The reliability of this study was very limited (Klimisch 3).

Results

No effect on food intake or body weight was seen up to 1000 ppm. One male and one female dog died after 59 and 113 days of exposure to 3000 ppm. Due to the small group size no statistical evaluation of organ weights was performed. However, the authors of the study concluded that no adverse effects on organ weights were seen,

whereas exposure above 330 ppm resulted in histopathological changes in the liver and pancreas and a significant increase in plasma alkaline phosphatase activity.

Summary and comparison with the CLP criteria

Adverse effects on male reproductive organs were reported in a reproductive/developmental toxicity screening study in rats and in six repeated dose toxicity studies in rats and one study in mice. The study in dogs was of limited reliability due to low number of animals tested.
In males, the results from the studies in rats, ranging from 28-days to 18-months exposure, consistently showed dose-related adverse effects on male sexual function and fertility following exposure to DBMC from approx. 40 mg/kg bw/d, including severe reduction in testes and epididymis weights, testis tubules atrophy, spermatogenic arrest and changes in sperm motility, viability and morphology. Similar effects were also reported in the mouse study with 2 months of exposure to DBMC. From 40 to 200 mg/kg bw/d no or moderate general toxicity was reported evident as reduction in body weight from 0-9% across the studies and relative liver weight increase from 0-30%. The reported effects on sexual function and fertility in males at these dose levels are therefore not considered to be secondary non-specific consequences of general toxicity. From 500 mg/kg bw/d marked general toxicity was reported including mortality and severe body weight loss, and large increases in liver weight. However, as effects on sexual function and fertility were reported in the absence of significant general toxicity in the studies with lower doses of DBMC, the effects on the male reproductive organ at higher doses are considered to be related to treatment and not as secondary consequences of general toxicity. A MoA for the adverse effects on male reproductive organs was suggested by Takagi *et al*. (1994) and consisted of an uncoupling action in the mitochondria, leading to an inhibition of the energy production in cells, resulting in a lack of ATP, which is necessary for cell division. This possible MoA could severely affect testes as an organ of a very high level of cell division and consequently a high energy consumption. However, no experimental data is available to support this MoA hypothesis, to exclude other MoAs or to indicate that the MoA would not be relevant to humans. Therefore, RAC considers that the observed effects on sexual function and fertility are relevant to humans. Further, RAC consider that the clear effects on testicular function warrants classification, despite very limited effects on pup production in the reproductive/developmental screening study. This is considered related to the fact that rats have an enormous excess of spermatozoa in their ejaculates, so that sperm counts has to be reduced by as much as 90% to affect fertility (Mangelsdorf *et al*., 2003) leading to a situation where a reduction in sperm count may not result in reduced fertility, especially in rodent studies.

In females the effects on the ovary, uterus, number of corpora lutea and implantation scars reported following exposure to DBMC in some of the repeated dose toxicity studies and in the reproductive/developmental screening study were inconclusive, as they occurred sporadically across the available studies. However, the findings are considered as supportive evidence of the adverse effects on sexual function and fertility.

In conclusion, RAC agrees with the DS that based on the adverse effects consistently reported in the male reproductive organs in one reproductive/developmental toxicity screening study in rats, and in several repeated dose toxicity studies in rats and one in mice, which are considered not to be secondary non-specific consequence of other toxic effects, **classification as Repr. 1B; H360F for adverse effects on fertility** is warranted.

Setting of Specific Concentration Limits

The DS proposed that no SCL was warranted for effects on sexual function and fertility and that the GCL should be applied. This was based on results from the reproductive/developmental screening study and the repeated dose toxicity studies. A precise ED_{10} was not readily established by the DS based on the results of these studies. However, it appeared that the ED_{10} would probably be between the NOAEL and the LOAEL of around 12.5 – 40/88 mg/kg bw/d, which would indicate that DBMC should be placed in the medium potency group (4 mg/kg bw/d < ED_{10} > 400 mg/kg bw/d). Therefore, RAC agrees with the DS that no SCL is warranted for sexual function and fertility.

Developmental toxicity

The DS included for the assessment of developmental toxicity two studies in rats; a developmental toxicity study (no OECD TG but similar to 414, no GLP) and the reproduction/developmental toxicity screening study (OECD TG 421 and GLP).

In the prenatal developmental toxicity study in Wistar rats (Tanaka *et al*., 1990), female rats were exposed to 0, 93.5, 187.0 or 375.0 mg/kg bw/d DBMC by gavage on GD 7-17.

Results

In the low dose group, no adverse effects were reported in dams or offspring. In the mid-dose group, some signs of general toxicity in dams including diarrhoea, hair fluffing, suppression of body weight (approx. 6% on GD 20) and supressed food consumption was noted, but no effects on the number of implantations or corpora lutea and no effects in the offspring were reported. In the high-dose group, two (2/22=9.1%) dams died during the study. The same general toxicity in dams was reported as in the mid-dose group, however, the suppression of body weight was approx. 16%. No effects were recorded on the number of implantations or corpora lutea. However, a statistically non-significant increase in the number of dead implants (28.4% prenatal mortality vs. 8.6% in controls) and in dams with only dead implants (25% vs. 4.2% in controls) was reported (see table below) at the top dose leading to a low number of litters (15) examined for malformations as compared to controls litters (23). No malformations or variations were reported. RAC notes that the mortality reported in the study (9.1%) is close to 10%, a level which in the CLP Guidance is considered to indicate excessive maternal toxicity and hence the data for such dose levels shall not normally be considered for further evaluation. Therefore, the increase in the number of dams with dead implants reported in the high dose group are considered to be secondary, non-specific consequences of maternal toxicity.

Table: *effects of DBMC to pregnant rats on foetal development*

Dose (mg/kg bw/d)	**0**	**93.5**	**187.0**	**375.0**
# dams	24	20	20	20
# live foetuses	273 (11.4 ± 3.4)	288 (14.4 ± 2.0*)	264 (13.2 ± 1.7)	180 (9.0 ± 5.7)
# dead implants Early death Late death Mortality (%)	26 20 6 8.6	12 12 0 4.1	7 7 0 2.4	69 68 1 28.4
# dams with dead implants only (%)	1 (4.2)	0	0	5 (25)

In the reproduction/developmental toxicity screening study, CD (SD) rats were exposed to 0, 12.5, 50, 200 and 800 mg/kg bw/d DBMC by gavage (females from 14 days before mating to lactation day 3).

Results

Up to 200 mg/kg bw/d no effects on dams or offspring were reported. At 200 mg/kg bw/d, general toxicity in dams included a statistically significantly reduced corrected body weight (7%) at PND 4 and reduced body weight gain (11%) at termination of lactation compared to controls. In addition, a statistically significantly lower food consumption was seen periodically during pre-mating, pregnancy and lactation. A statistically significant decrease in pups born, delivery index, live pups born and live pups on lactation day 4 was reported, see table below. At 800 mg/kg bw/d general maternal toxicity included a decrease in maternal body weight (6%) as well as the corrected body weight (8%) compared to controls at pregnancy day 21. At necropsy the corrected body weight was 9% lower than in controls. The weight gain was decreased (by 30%) from study start until termination on lactation day 3 compared to control animals. Food consumption was periodically lower during premating, pregnancy and lactation, and occasional cases of loose stools and salivation were reported, however, no effect was seen in 9/12 animals. A significant decrease in the numbers of corpora lutea and implantation scars were reported (see table below). One dam was unable to deliver pups, and another dam lost all pups during lactation. A small but significant decrease in the number of pups born was also reported. The birth weights of offspring were approx. 10% decreased compared to control pups. However, no clear dose-response was seen regarding the number of pups born, the delivery index as well as live pups on lactation day 4. Decreased body weight gain, although moderate during pregnancy, may indicate maternal toxicity. However, without information on the correlation of body weight with these effects on the basis of individual animal data, it is difficult to conclude whether this is a secondary effect. Overall, RAC considers these effects to be of limited biological relevance for classification according to the CLP criteria.

Table: *Effects of DBMC on foetal toxicity*

Dose (mg/kg bw/d)	0	12.5	50	200	800
# dams	12	12	12	12	12
Corpora lutea	16.4 ± 3.0	16.4 ± 2.6	16.3 ± 1.5	15.1 ± 1.4	14.1 ± 1.6*
Implantation scars	14.3 ± 3.0	14.7 ± 1.1	15.2 ± 1.3	13.5 ± 1.4	13.1 ± 1.5*
Implantation index (%)	86.9 ± 14.7	91.0 ± 12.8	93.6 ± 7.4	90.1 ± 8.5	93.1 ± 7.2
Gestation index (%)	100	100	100	100	83.3
Pups born	13.5 ± 3.3	13.5 ± 1.0	14.8 ± 1.3	11.7 ± 1.4**	12.2 ± 1.8*
Delivery index (%)	93.5 ± 8.9	92.2 ± 5.2	97.3 ± 3.3	87.2 ± 10.5*	92.8 ± 5.7
Live pups born	13.1 ± 3.2	13.3 ± 0.8	14.3 ± 1.3	11.5 ± 1.0**	11.3 ± 4.1
Live pups lactation day 4	13.1 ± 3.2	13.3 ± 0.8	14.3 ± 1.4	11.4 ± 1.0**	12.4 ± 1.8 (10 dams)

*p<0.05, **p<0.01

In summary, the results from the two studies with DBMC assessing developmental toxicity do not indicate any teratogenic effects of DBMC. In the developmental toxicity study with rats a statistically non-significant decrease in the number of liveborn foetuses was reported at 375

mg/kg bw/d. However, at the same dose level, two (9.1%) dams died showing that foetal toxicity was reported at doses with marked maternal toxicity. In the screening reproduction/developmental study foetal toxicity was seen in the two high dose groups (200 and 800 mg/kg bw/d) with a reduced number of live foetuses. However, the effects were reported without any clear dose-response relationship.

Overall, RAC concludes that the decrease in the number of liveborn foetuses/pups reported are secondary non-specific consequences of maternal toxicity following exposure to DBMC, and agrees with the DS that **no classification for developmental toxicity** is warranted.

In conclusion, RAC agreed in line with the DS that DBMC should be **classified as Repr. 1B; H360F for adverse effects on fertility.**

10.11 Specific target organ toxicity-single exposure

Not evaluated.

10.12 Specific target organ toxicity-repeated exposure

The end-point of specific target organ toxicity-repeated exposure is not evaluated.

The effects seen in the numerous repeated dose toxicity studies show that the target organs of DBMC are the male and the female reproductive organs. The studies are therefore described under point 10.10 above.

10.13 Aspiration hazard

Not evaluated.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated.

13 ADDITIONAL LABELLING

Not evaluated.

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ANNEXES

ANNEX I

Standardised summaries of the individual studies (separate document)

ANNEX II: Weight of evidence analysis of adverse effects on sexual function and fertility (separate document)