Committee for Risk Assessment

RAC

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

Adopted

13 June 2019

13 June 2019

CLH-O-0000001412-86-288/F

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

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

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

EC Number: **204-327-1**

CAS Number: **119-47-1**

The proposal was submitted by **Denmark** and received by RAC on **31 July 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

Denmark has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **8 October 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **7 December 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Christine Bjørge**

Co-Rapporteur, appointed by RAC: **Ruth Moeller**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **13 June 2019** by **consensus**.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	604-RST-VW-Y	6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol; [DBMC]	204-327-1	119-47-1	Repr. 1B	H360F	GHS08 Dgr	H360F			
RAC opinion	604-RST-VW-Y	6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol; [DBMC]	204-327-1	119-47-1	Repr. 1B	H360F	GHS08 Dgr	H360F			
Resulting Annex VI entry if agreed by COM	604-RST-VW-Y	6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol; [DBMC]	204-327-1	119-47-1	Repr. 1B	H360F	GHS08 Dgr	H360F			

GROUNDS FOR ADOPTION OF THE OPINION

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).

HUMAN HEALTH HAZARD EVALUATION

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.**

Additional references

Mangelsdorf I, Buschmann J and Orthen B. 2003. Some aspects relating to the evaluation of the effects of chemicals on male fertility. Reg. Tox. Pharm. 37, 356-369.

ANNEXES:

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

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