

Annex II: Weight of Evidence analysis (WoE)

1. PROBLEM FORMULATION

A weight of evidence approach to conclude on the classification of 6,6'-di-tert-butyl-2,2'-methylenedi-p-cresol (DBMC) for the endpoint fertility according to the CLP criteria.

The approach considers the following elements:

- Specific effects on male reproductive functional parameters (incidence, severity, dose response and temporal concordance)
- Specific effects on male reproductive organs (incidence, severity, dose response and temporal concordance)
- Reversibility
- Human Relevance

2. Collection and documentation of all available evidence (search strategy)

The substance has been evaluated under the process of Substance Evaluation. The data on systemic toxicity available within the Registration dossier has been used in the assessment.

The search in eChemportal identified that the substance has been assessed under the OECD SIDS programme (https://hpvchemicals.oecd.org/UI/SIDS_Details.aspx?id=984ED015-F315-46E2-961D-C5BC83682451) and by NICNAS (human health tier II assessment: https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-assessment-details?assessment_id=459).

The data sets used in these assessments were identical to the ones used in the current WoE analysis presented in this section.

In addition, experimental data retrieved from the public domain has been used, based on a limited literature search performed using the name of the substance in Pubmed. This search in Pubmed did not yield any additional information on DBMC, regarding adverse effects on sexual function and fertility, but a few studies on structural analogues were found.

The experimental data available used are reported in terms of quality in Section 3.

The QSAR Toolbox has been used to identify potential analogues or data with the substance in support of the WoE approach. Information on one analogue, 6,6'-Di-tert-butyl-4,4'-diethyl-2,2'-methylenediphenol, CAS 88-24-4, is considered in the quality assessment (Section 3) and integration of evidence (section 4.2).

3. Assessment of quality of individual evidence

A summary of the assessment of the quality of each line of evidence/study is provided in the table below. The criteria defined within ECHA Guidance R.4 have been used to assess

relevance, reliability and adequacy. The quality of each line of evidence (in relation to reliability, adequacy and relevance) is further assessed in the integration of evidence step.

The majority of the studies were of good quality, and the reporting permitted conclusion on the effects seen in the testes and on sperm.

Some of the studies had limitations due to the absence of measurement of sperm related parameters in addition to the histopathological testicular effects reported. This did not allow a direct conclusion whether histopathological testicular effects occur prior or after the sperm effects within these specific studies. Some limitations in reporting were identified. These were overcome by using the robust study summaries available in the Registration dossier, and in the OECD SIDS together with the original publications for the assessment of the findings. ch.

The limitations are reflected in summary within the adequacy column and further reflected with the Klimisch score. The latter is only indicative of the quality of the evidence at this stage, and all information is taken forward for further assessment in section 4.

Klimisch score 1 (reliable without restrictions) is assigned to Study 1 which was performed according to OECD test guideline 421 and according to GLP.

Klimisch score 2 (reliable with restrictions) is assigned to Studies 2-7, as they were either non GLP or did not totally comply with a specific test guideline but were sufficient to accept the data and were well documented and scientifically acceptable.

Klimisch score 3 (unreliable) is assigned to Study 8, due to the low number of animals used that can be considered as to have been carried out according to a method that is not acceptable (statistical power of the results is limited).

Studies 1, 2, 3, 4 and 7 had also been assessed under OECD SIDS and by the Registrant and the same reliabilities have been concluded as presented in the current WoE analysis.

Definite considerations regarding the quality of all available evidence is performed at the integration step using additional elements (consistency, specificity and plausibility). This takes into account the fact that some studies were non-GLP (e.g. study 2 and study 3), which does not affect their quality on the basis of consistency and specificity as described in section 4. The use of the Klimisch scoring is one indicator of the quality of evidence and does not predefine the acceptance or exclusion of a study prior to integration of evidence.

The details of the assessment for each study are available within the CLH report and Annex I of the CLH report.

The following table shows the assessment of the quality of individual evidence (assessment of male functional and organ parameters) per study.

Relevance: All studies have been identified as relevant taking into account that: the substance tested was representative for the substance as being assessed, appropriate species have been studied, the route of exposure is relevant for the population, appropriate doses/concentrations have been tested, critical parameters influencing the endpoint have been considered adequately.

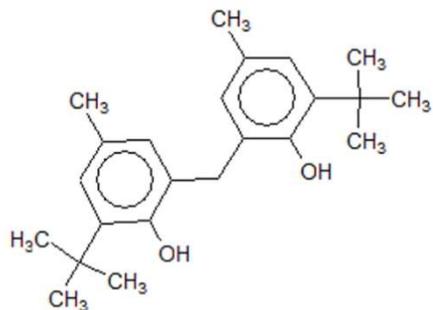
Reliability: Klimisch score has been used in this step for preliminary assessment of reliability.

Adequacy: was assessed regarding usefulness of information for the purpose of classification for reproductive toxicity, taking into account the weight attached to the studies that are the most relevant and reliable.

CLH REPORT FOR 6,6'-DI-*TERT*-BUTYL-2,2'-METHYLENEDI-*P*-CRESOL (DBMC)

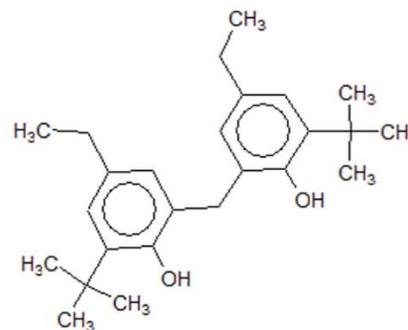
Experimental Studies with DBMC, CAS 119-47-1					
Type of Evidence / Data Source - Reference	Exposure duration	Species / Route / Administration /	Relevance	Reliability	Adequacy
Study 1 OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test), GLP/ Ministry of Health and Welfare Japan (1999b)	52 days	Rat (Crj: CD(SD)) / Oral / Gavage /	Relevant in WoE (route, species, parameters)	Klimisch Score: 1	Adequate for the assessment of male fertility
Study 2 Subchronic oral toxicity study, not GLP. Takagi et al. 1994	28days and 90days (12 weeks)	Rat (Wistar) / Oral / Feed /	Relevant in WoE (route, species, parameters)	Klimisch Score: 2	Adequate for the assessment of male fertility
Study 3 Chronic oral toxicity study, not GLP. Takagi et al. 1994	6, 12, 18 months	Rat (Wistar) / Oral / Feed /	Relevant in WoE (route, species, parameters)	Klimisch Score: 2	Adequate for the assessment of male fertility
Study 4 Subacute toxicity study Ministry of Health and Welfare Japan (1996a)	28 days	Rat (Crj: CD(SD)) / oral: capsule	Relevant in WoE (route, species, parameters)	Klimisch Score: 2	Adequate for the assessment of male fertility
Study 5 2 month toxicity study, Takahashi et al. 2006	60days	Male rats (F344/DuCrj (Fischer) / Oral / Feed Male mice (Crj: CD(ICR) / Oral / Feed	Relevant in WoE (route, species, parameters)	Klimisch Score: 2	Limitations: Only one dose used; information considered in the integration of the evidence
Study 6 Subchronic toxicity study (13weeks), Bomhard et al. 1982	90days	Rat (Wistar) / Oral / Feed	Relevant in WoE (route, species, parameters)	Klimisch Score: 2	Limited information on test conditions and parameters measured. Considered in the integration of the evidence
Study 7 Sub-chronic toxicity study, American Cyanamid Company 1965	90days	Rats (Nelson strain albino rats) / Oral / Feed	Relevant in WoE (route, species, parameters)	Klimisch Score: 2	Adequate for the assessment of male fertility; no measurements on sperm parameters reported.
Study 8 Sub-chronic toxicity study, Dog	90days	Dog (Beagle) / Oral / Feed	Relevant in WoE (route, species, parameters)	Klimisch Score: 3	Only 2 dogs per sex per dose used in the study reducing the statistical power and relevance.

Information on analogue substance (2,2'-methylenebis (4-ethyl-6-*tert*-butylphenol), CAS number 88-24-4.



DBMC:

6,6'-di-*tert*-butyl-2,2'-methylenebis-*p*-cresol, CAS: 119-47-1



diethyl analogue to DBMC:

2,2'-methylenebis (4-ethyl-6-*tert*-butylphenol), CAS: 88-24-4

CLH REPORT FOR 6,6'-DI-*TERT*-BUTYL-2,2'-METHYLENEDI-*P*-CRESOL (DBMC)

Information on analogue substance (2,2'-methylenebis (4-ethyl-6-tert-butylphenol), CAS number 88-24-4.				
Type of Evidence / Data Source - Reference	Species / Route / Administration	Relevance	Reliability	Adequacy
Takagi et al (1994): Acute, Subchronic and chronic toxicity study with 2,2-isobutylidenebis (4,6-dimethylphenol) in rats,	Rat (Wistar) / Oral / Feed	Relevant in WoE (route, species, parameters)	Klimisch Score: 2	Adequate for the assessment of male fertility
Takagi, A., Momma, J., Aida, Y., Takada, K., Suzuki, S., Naitoh, K., Tobe, M., Hasegawa, R and Kurokawa, Y. (1992): Toxicity studies of a synthetic antioxidant, 2,2'-methylenebis (4-ethyl-6-tert-butylphenol) in rats. Acute and subchronic toxicity. J. Toxicol. Sci., 17, 135-153	Rat	Relevant in WoE (route, species, parameters)	Klimisch Score: 2	Adequate for the assessment of male fertility
Takagi, A., Takada, K., Sai, K., Momma, J., Aida, Y., Suzuki, S., Naitoh, K., Tobe, M., Hasegawa, R. and Kurokawa, Y. (1996): Chronic oral toxicity of a synthetic antioxidant, 2,2'-methylenebis(4-ethyl-6-tert-butylphenol), in rats. J. Appl. Toxicol., 16, 15-23	Rat	Relevant in WoE (route, species, parameters)	Klimisch Score: 2	Adequate for the assessment of male fertility

The information from the diethyl analogue is not considered in the dose-response and temporal concordance analysis but only in the section of Integration of evidence, in particular for the derivation of confidence and assessment of remaining uncertainty and plausible mode of action for the effects observed. The details from these studies are available via the corresponding publications.

4. Integration of evidence and overall weighing of evidence

4.1 Dose-response and temporal concordance

The analysis of available evidence from the screening reproductive toxicity study and the repeated dose toxicity studies are analysed in view of time (exposure periods) and doses of the registered substance. The dose-response and temporal concordance observed is used as one of the elements of consistency and specificity in integrating the evidence and weighing them.

The data for the dose-response and temporal concordance analysis, regarding effects on sperm and testes, respectively, summarise the results on fertility and sexual function of the respective studies as reported in Annex I of the present CLH report.

In order to represent the various sperm and organ related parameters in a single tabular format, some approximation of the results was required, using the number of symbols from one to three to reflect severity and incidence.

To discriminate between effects from single studies each study is given a symbol in the dose-response temporal concordance tables (see list below).

"(no effects)": no effects were observed in a study at a specific tested dose, this is indicated within the corresponding field in the table.

(-): no measurements were performed or reported within a study or when the dose was not tested for a study

□ Study 1:

Screening reproductive toxicity study (52 days) indicates a dose-response effect on male reproductive system starting at 50 mg/kg bw/day for sperm related parameters and at 200 mg/kg bw/day for male reproductive organ histopathological effects with increasing concentrations causing increasing severity and incidence. The NOAEL in this study was 12.5 mg/kg bw/day.

■ Study 2:

28-day (4 weeks) exposure resulted in sperm related parameters effects in a dose response manner starting at 88 mg/kg bw/day. Male reproductive organ histopathological changes occurred at 564mg/kg bw/day and higher (non-significant testicular weight changes occurred at 88 mg/kg bw/day).

X Study 2:

90-day exposure resulted in both sperm and male reproductive organ histopathological changes starting at 88 mg/kg bw/day in a dose-response manner.

▲ Study 3:

At 6-18 months exposure periods male reproductive effects occur at the highest dose tested (50 mg/kg bw/day).

+ Study 4:

28 day repeated dose toxicity study. Dose response is present for both sperm related parameters and pathological effects in male reproductive organs. The results indicate earlier

effect on sperm related parameters (occurring at 50 mg/kg bw/day) with no NOAEL found for this effect - compared to pathological effects in male reproductive organs (occurring at 200 mg/kg bw/day). The results from the 14-day recovery period showed that the adverse effects on male reproductive organs structure and function had not yet recovered after 14 days without exposure and indicate that the effects may be irreversible.

Study 5:

2-month study with mice & rat – the study results are not included in the tables as only one dose level is used. In rats, the dose of 40-60 mg/kg bw/day caused adverse effects on both sperm parameters and testes histopathology. In mice the single dose level of 414 mg/kg bw/day caused effects on testicular and sperm parameters.

○ Study 6:

Severe male reproductive organ toxicity (testicular weight and atrophy) in a dose-response manner at concentrations above 75.65 mg/kg bw/day; sperm related parameters are not reported in the study, and the testicular effects description is limited.

● Study 7:

Male reproductive organ histopathological adverse effects observed at 80.3 and 241 mg/kg bw/day; there was no specific reporting on sperm parameters (therefore the study is not included in the first table below).

Study 8:

The repeated dose toxicity study in dogs (Study 8) is not considered in the dose response and temporal concordance tables below due to limitations of the study design (low number of animals tested). The information from this study is assessed within the integration of the evidence (as part of the consistency assessment).

The effects on sperm related parameters and male reproductive organs were grouped from each study. The type of parameter or male reproductive organ effect per study are outlined in the table below:

	Sperm Measured Parameter	Male Reproductive Organ Histopathology
Study 1 (52 days)	Sperm Motility Ratio Sperm Viability Ratio Sperm Survivability Ratio Amount of Sperm in Epididymis Cauda Abnormal Sperm Ratio	Absolute and Relative testis weight Absolute and Relative epididymis weight Seminiferous tubules atrophy Testis: Giant cell formation
Study 2 (28 day)	Decreased Spermatogenesis Spermatogenic arrest Hypospermia	Absolute and Relative testis weight Testis: Giant cell formation Testis: Interstitial edema Seminal vesicles and prostate atrophy
Study 2 (90 days)	Decreased Spermatogenesis Spermatogenic arrest Hypospermia	Absolute and Relative testis weight Testicular tubule atrophy Testis: Giant cell formation Testis: Interstitial edema Seminal vesicles and prostate atrophy
Study 3 (6-18months)	Decreased Spermatogenesis Spermatogenic arrest Hypospermia	Testicular tubule atrophy
Study 4 (28 day)	Spermatid degeneration Sperm retention Spermatid nuclear vacuolation Germ cells decrease	Sertoli cells vacuolation Giant cell formation
Study 6 (90 days)		Absolute and Relative testis weight Testicular Atrophy
Study 7 (90 days)		Testicular Atrophy

CLH REPORT FOR 6,6'-DI-*TERT*-BUTYL-2,2'-METHYLENEDI-*P*-CRESOL (DBMC)

Increasing exposure duration causes similar or increasing sperm toxicity

Male reproductive Parameter Effects (Effect in relation to sperm measured parameters)						
Time	28 days (study 2&4)	52 days (study 1)	90 days (study 2)	6months (study 3)	12months (study 3)	18months (study 3)
Dose Ranges (mg/kg bw/day)						
0	(no effects)	(no effects)	(no effects)	(no effects)	(no effects)	(no effects)
4 (7.41)	-	-	-	(no effects study 3)	(no effects study 3)	(no effects study 3)
12.5 (12.7)	-	(no effects)	-	(no effects study 3)	(no effects study 3)	(no effects study 3)
25 (24.91)	-	-	-	-	-	-
50 (42.3; 30; 38.6-58))	+	□□		▲▲▲	▲▲▲	▲▲▲
75.65	-	-	-	-	-	-
88 (80.3)	■ ■	-	△△	-	-	-
200 (241)	++	□□□	-	-	-	-
281.64	-	-	-	-	-	-
564	■ ■ ■	-	△△△ **	-	-	-
800	++	□□□	-	-	-	-
3120	■ ■ ■ **	-	△△△ **	-	-	-

** : Presence of marked systemic toxicity (mortality)

Increasing doses cause similar or increasing or sperm

Increasing exposure duration causes increasing or similar male reproductive organ toxicity

Male reproductive Organ Effects (Histopathology findings)						
Time	28 days (study 2&4)	52 days (study 1)	90 days (study 2,6,7)	6months (study 3)	12months (study 3)	18months (study 3)
Dose Ranges (mg/kg bw/day)						
0	(no effects)	(no effects)	(no effects)	(no effects)	(no effects)	(no effects)
4.23 (7.41)	-	-	(no effects study 6)	(no effects study 3)	(no effects study 3)	(no effects study 3)
12.5 (12.7)	-	(no effects)	-	(no effects study 3)	(no effects study 3)	(no effects study 3)
25 (24.91)	-	-	(no effects study 6, 7)	-	-	-
50 (42.3; 30; 38.6-58)		(no effects)		▲▲▲	▲▲▲	▲▲▲
75.65	-	-	○○	-	-	-
88 (80.3)		-	●● ▲▲	-	-	-
200 (241)	++	□□	●●●	-	-	-
281.64	-	-	○○	-	-	-
564	■ ■	-	** ▲▲▲	-	-	-
800	++	□□□	-	-	-	-
31201	** ■ ■ ■	-	** ▲▲▲	-	-	-

Increasing dose causes increasing or similar male reproductive organ toxicity

** : Presence of marked systemic toxicity (mortality)

As shown in the tables above, overall increasing incidence and severity occurs with increasing doses and increasing exposure time, with some expectable biological variability in response incidence and severity between different tests of the same duration. The effects are specific in nature, as in the majority of the doses there is no other severe relevant systemic toxicity observed in the male rodent species (unless otherwise specified). There was increased mortality observed in Study 2, (both at 28 and at 90 day duration of exposure as indicated in the dose-response temporal concordance tables). In these cases, the observed adverse effects on male reproductive parameters and organs are considered within the dose-response analysis to understand if there is a pattern for this type of effect although the effect is present at high doses that causes marked systemic toxicity.

According to the CLP Guidance on Parental toxicity, "Adverse effects on sexual function and fertility seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute bw, coma) are not relevant for classification."

The adverse effects on male reproduction are not seen only at dose levels causing marked systemic toxicity in Study 2, but effects are observed also at doses before the initiation of the marked systemic toxicity.

There is some indication that the effects on sperm parameters (functional male fertility measure) occur prior to effects on male reproductive organs when comparing the results from the two tables for the studies where both sperm parameters and male reproductive organs were examined within the same study (sperm related effects at 50 and 88mg/kg bw/day at 28 and 52 day exposure periods in absence of any male reproductive organ histopathological finding).

4.2 Consistency – Specificity / Biological Plausibility

The following table demonstrates that the available evidence shows high **consistency and specificity**: All the studies in rodent species show lead toxicity towards the male reproductive system (sperm, testes). There are indications from the dose-response and temporal concordance that the parameters related to the male functional fertility are affected prior to those related to structural alterations in male reproductive organs, specifically the 28-day repeated exposure showing effects on sperm parameters at lower exposure doses than those required to elicit a testicular effect.

There is limited information from all studies regarding reversibility. One 28 day repeated dose toxicity study with a 14-day recovery period allows to conclude that within the tested time frame the effects are irreversible in that study.

The information from the **analogue** diethyl-(2,2'-methylenebis (4-ethyl-6-tert-butylphenol, CAS 88-24-4) also shows similar toxicity profile for testicular toxicity increasing the confidence levels in the assessment. The analogue has also caused atrophy in testicular tubules and decrease of spermatogenesis in sub-chronic toxicity studies. No effects on male reproduction were observed with low concentrations in a chronic study with DEMC; this is in line with the observation from the toxicological data set for the substance where no effects are identified below 50mg/kg bw/day (Takagi et al, 1996, Takagi et al, 1992). Additional considerations as reported by Takagi (Takagi et al, 2005) provide indication about the structural elements that might be responsible for the testicular toxicity of some phenolic antioxidants (tert butyl substitution at -6 of phenol, two phenolic groups).

A possible **mode of action**, suggested by Tagaki et al. (1994), is the molecular mechanism of uncoupling in mitochondria. Tagaki et al. (1994) showed that DBMC, and a structurally similar anti-oxidant (2,2'-methylenebis (4-ethyl-6-tert-butylphenol) (MBEBP) CAS no 88-24-2) exert

an uncoupling action in isolated liver mitochondria. 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 than 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.

The conclusions on **confidence** for each line of evidence (findings from each study used) and remaining uncertainty is derived taking into account the quality of the data assessed in the previous section, the consistency and specificity (in terms of incidence, dose response and temporal concordance) and information from similar substance. The principles of deriving confidence levels qualitative as described in SCHENHIR "Memorandum on the use of the scientific literature for human health risk assessment purposes, weighing of evidence and expression of uncertainty" and in line with the principles of the OECD AOP User Guidance Manual¹, have been used.

Explanatory notes for the table below:

Consistency and Specificity: Refers to the evidence available. For each type of evidence consistency and specificity is addressed showing that all the evidence points to the same direction (positive effects in male reproductive parameters)

Likelihood / Biological Plausibility: Refers to the general biology principles that effects to male reproductive parameters are linked to infertility.

It is noted that the screening reproductive toxicity study, despite the clear effects reported on sperm and males reproductive organs, does not show an effect in fertility index. However, as per OECD Guidance on mammalian reproductive toxicity testing and assessment: *"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. A reduction in sperm count may not result in reduced fertility, particularly in rodent studies. This is due to 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. It is important to note that sperm concentrations in human males are highly variable and generally lower than in rodents. The distribution of counts is 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."* (OECD, 2008).

Temporality: There is a logical order in the occurrence of an effect on the basis of the evidence available as indicated in the dose-response temporality table in the previous section.

Confidence levels are derived taking into account the outcome of the weighing of the evidence (both individually and collectively) using the metrics/criteria specified in the corresponding steps of the WoE approach (such as adequacy, relevance, reliability for individual evidence

¹ High confidence level: extensive evidence for dose-response and temporal concordance, no inconsistent evidence in literature, more studies showing the same effect, Medium confidence level: some inconsistent effects but can be explained, Low confidence level: inconsistent with remaining evidence, quality of data low

assessment, and consistency/specificity, plausibility/likelihood, temporality for WoE analysis). Confidence levels are expressed as high, medium or low. The confidence levels for each line of evidence feed to the judgement of the overall confidence level that takes into account all the evidence in an integrated and weighed mode. For each line of evidence confidence levels have as underlying documentation:

1. Qualitative elements (e.g. Likelihood/ Biological plausibility)
2. Semi (quantitative) elements (e.g. temporality)

The level of confidence of each line of evidence is derived by combining the quality assessment elements of each line of evidence (relevance, adequacy, reliability) with the consistency and plausibility elements.

CLH REPORT FOR 6,6'-DI-*TERT*-BUTYL-2,2'-METHYLENEDI-*P*-CRESOL (DBMC)

Type of Evidence	Consistency & Specificity	Likelihood/ Biological Plausibility	Temporality	Human Relevance	Confidence / Strength of Evidence	Remaining Uncertainty
Similar to OECD Guideline 421	Consistent effects on male reproductive system (in the absence of other systemic toxicity)	Effect observed plausible to affect fertility. Also plausible for human relevance.	Yes, increasing time and doses produce an increase in adverse effects for male reproductive functional parameters & male reproductive organs	Yes	High	Low
Subchronic oral toxicity study, Takagi et al. 1994	Consistent effects on male reproductive system (in the absence of other systemic toxicity)	Effect observed plausible to affect fertility. Also plausible for human relevance.	Yes, increasing time and doses produce an increase in adverse effects for male reproductive functional parameters & male reproductive organs	Yes	High	Low
Chronic oral toxicity study	Consistent effects on male reproductive system (in the absence of other systemic toxicity)	Effect observed plausible to affect fertility. Also plausible for human relevance.	Yes, increasing time and doses produce an increase in adverse effects for male reproductive functional parameters & male reproductive organs	Yes	High	Low
Subacute toxicity study Ministry of Health and Welfare Japan (1996a)	Consistent effects on male reproductive system (in the absence of other systemic toxicity)	Effect observed plausible to affect fertility. Also plausible for human relevance.	Yes, increasing time and doses produce an increase in male reproductive functional parameters	Yes	High	Low
2 month toxicity study, Takahashi et al. 2006	Consistent effects on male reproductive system (in the absence of other systemic toxicity)	Effect observed plausible to affect fertility. Also plausible for human relevance.	Not available	Yes	Medium: one dose used in mice & rat consistent findings with the rodent assays	Low
Subchronic toxicity study (13 weeks), Bomhard et al. 1982	Consistent effects on male reproductive system (in the absence of other systemic toxicity)	Effect observed plausible to affect fertility. Also plausible for human relevance.	Yes, increasing time and doses produce an increase in adverse effects for male reproductive organs	Yes	High	Low
Sub-chronic study, American Cyanamid Company 1965	Consistent effects on male reproductive system (in the absence of other systemic toxicity)	Effect observed plausible to affect fertility. Also plausible for human relevance.	Yes, increasing time and doses produce an increase in adverse effects for male reproductive organs	Yes	High	Low
Sub-chronic toxicity study, Dog	Inconsistent; absence of effects in male dog reproductive system	Not plausible	N/A	Cannot be determined; inadequate study.	Low; on the basis of quality of the study	N/A

Overall consistency and specificity: The available evidence, when considering the quality of the data, the dose-response and temporal concordance, the same type of effect across studies and the irreversibility (when study examined it) shows high consistency and specificity for effect to male reproductive system and its functional parameters.

Temporality and Dose-response relationship: At each individual experimental repeated dose toxicity study increasing doses cause increase incidence and severity in male reproductive parameters.

Incidence and severity in male reproductive parameters increase with time and dose across experimental data.

Human Relevance: Yes, the effects observed are specific to the male reproductive system (fertility parameters and organ specific toxicity). In absence of information on species differences in toxicokinetics between animals and humans, no contradictive evidence from reliable studies in other species, no specific mode of action in animals identified that would allow conclusion for non-human relevance, the available evidence is considered sufficient to conclude relevance for humans and qualitative concordance across species. The available negative dog study is of low overall quality and statistical power due to a low number of animals used and cannot overwrite the findings from multiple rodent repeated dose toxicity studies that evaluated the male reproductive system.

Conclusion from overall confidence: High confidence on the basis of consistent and specific effects to male reproductive function with dose-response and temporal association from adequate and relevant experimental studies in rodents. The high level of concordance and specificity reduce any remaining uncertainty due to some limitations regarding the reporting of some of the studies.

5. Description of Uncertainty

The individual uncertainty sources from each study are reported in the details of each study and summarised in the assessment of the evidence and the integration of evidence by means of derivation of confidence levels.

Although some limitations in the available reporting of the studies exists (e.g. Japanese report with data available in English in tables) the available information is sufficient to conclude due to the overall concordance and specificity for the male reproductive effects (functional and organ) as presented in the previous sections.

Although a generation study is not available this is not considered a limiting factor to conclude for the purpose of classification for reproductive toxicity. Biological plausibility is high in terms of linking the consistent and specific clear adverse effects of DBMC to male gonads in rats in several studies to subsequent male infertility for humans.

6. CONCLUSIONS

The substance causes specific effects on male reproductive functional parameters and specific effects on male reproductive organs that are irreversible under the conditions of the available

studies. The effects occur in a dose-response relationship, and a temporal concordance appears across the studies.

No conclusions on the mode of action regarding the observed male reproductive system impairment in rodents can be drawn based on the available toxicological studies.

The adverse effects on the male reproduction organs and on sperm are considered relevant for humans.

The WoE analysis leads to the conclusion that the available data on DBMC provide clear evidence of human relevant specific adverse effects on sexual function and fertility which are not secondary to other toxic effects. Thus DBMC meets the criteria for classification for male reproductive fertility in category 1B.

7. REFERENCES

OECD (2008). Guidance document on mammalian reproductive toxicity testing and assessment. OECD Environment, Health and Safety Publications. Series on Testing and Assessment. No. 43, 2008).

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