SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

2,2'-dimethyl-4,4'-methylenebis(cyclohexylamine)

EC No 229-962-1

CAS RN 6864-37-5

Evaluating Member State(s): Germany

Dated: 24 March 2022

Evaluating Member State Competent Authority

BAuA

Federal Institute for Occupational Safety and Health

Division 5 - Federal Office for Chemicals

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Year of evaluation in CoRAP: 2015

Before concluding the substance evaluation a Decision to request further information was issued on: 14 May 2018

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

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Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

The Substance, 2,2'-dimethyl-4,4'-methylenebis(cyclohexylamine) ("DMDC") was originally selected for substance evaluation to clarify concerns about:

- Potential endocrine disrupting properties.

During the evaluation the following additional concerns were identified:

- Reproductive Toxicity: Fertility including developmental neurotoxicity
- Potential PBT properties based on screening level data indicating the Substance is not readily biodegradable and therefore needs to be considered as persistent.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

A testing proposal on long-term toxicity to soil macro-organisms was evaluated by ECHA and a decision was issued on 27 July 2012.[2]

ECHA conducted a compliance check for DMDC and issued a decision on 05 November 2015 requiring an *in vitro* gene mutation study in bacteria and a pre-natal developmental toxicity study in rabbits, oral route.[3]

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the Substance has led the evaluating Member State (eMSCA) to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

Based on the available information, the assessment of the eMSCA concludes that DMDC would fulfil the criteria for classification as STOT RE 1 and potentially Repr. 2 or 1B.

[2] Decision on a testing proposal for DMDC from 26 July 2012: https://echa.europa.eu/documents/10162/066303ee-b224-5ec4-d8b5-3541859152ed

[3] Decision on a compliance check for DMDC from 05 November 2015: https://echa.europa.eu/documents/10162/3e228696-1b70-bec3-8840-987282a9aa58

This classification is stricter than both the existing CLP Annex VI entry for DMDC and the self-classification employed by the registrants of the Substance.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not applicable.

4.1.3. Other EU-wide regulatory risk management measures

Not applicable.

4.1.4. Restriction

Not applicable.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not applicable.

5.2. Other actions

Not applicable.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Table 2

FOLLOW-UP Follow-up action	Date for intention	Actor
CLH for Repr. and STOT (cf. section 7.9.10)	tbd	DE CA

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substance, 2,2'-dimethyl-4,4'-methylenebis(cyclohexylamine) ("DMDC") was originally selected for substance evaluation to clarify concerns about:

- Potential endocrine disrupting properties.

During the evaluation the following additional concerns were identified:

- Reproductive Toxicity (Fertility including developmental neurotoxicity)
- Potential PBT properties based on screening level data indicating that DMDC is not readily biodegradable.

The concerns have been assessed resulting in the outcome described in Table 4.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Endocrine disruption	Concern refuted. The eMSCA does not consider DMDC as an endocrine disruptor for human health and the environment.
Endocrine disruption (Human health)	Concern refuted. Based on the new information provided under substance evaluation and generated under compliance check, DMDC is not considered as an ED.
Endocrine disruption (Environment)	Concern refuted. Based on the assessment of structure-activity relationships, the available *in vitro* data and on the information from the provided mammalian studies, the eMSCA concludes that DMDC is not considered as an ED for the environment. Hence, the concern is clarified in a weight of evidence analysis and further testing is not necessary.
PBT properties	Concern refuted. The eMSCA does not consider the PBT or vPvB criteria, as laid down in Annex XIII of REACH, as fulfilled for DMDC.
Persistence	Concern unresolved. Based on the available information on degradation, DMDC may potentially fulfil the Annex XIII criterion for persistence (P).
Bioaccumulation	Concern refuted. Based on the estimation and experimental determination of the bioconcentration factor (BCF), DMDC does not fulfil the B criterion according to Annex XIII.
Toxicity	Concern unresolved. Based on available information, DMDC may fulfil the Annex XIII criterion for toxicity (T) based on the potential for classification as STOT RE 1 and/or Repr.

Toxicity to reproduction - fertility	Concern confirmed. Based on the observed effects in the available studies, DMDC is considered to meet the criteria for either suspected or presumed reproductive toxicant according to CLP (i.e. Repr. 2 or 1B). The classification needs to be adapted accordingly in a CLH process.
Toxicity to reproduction - developmental neurotoxicity	Concern unresolved. Based on the available findings DMDC does not exert specific neurodevelopmental toxicity (neuropathological findings were observed as well in adult animals). However, for some findings in neuro-behavioural testing, a developmental aetiology cannot be excluded.
Additional endpoints evaluated	**Outcome/conclusion**
Exposure	The substance is mainly used as a cross-linking agent in epoxy resins. It is expected that release from the uses of the substance as well as from articles to the environment are low. No further action necessary.
Toxicokinetics	The eMSCA does not concur with the registrants' conclusions on dermal absorption: 100 % absorption should be anticipated for the oral, inhalation and dermal uptake route. The registrations' chemical risk assessment should be updated.
Repeated dose toxicity	Based on results from repeated-dose toxicity studies, DMDC fulfils the criteria for classification as STOT RE 1. The self-classification should be adapted accordingly.

7.2. Procedure

The Substance was included on the Community Rolling Action Plan for substance evaluation on 26 March 2014. The substance evaluation process started on 17 March 2015.

The Substance was originally selected for substance evaluation to clarify concerns about its endocrine disrupting properties. PBT properties, toxicity to reproduction and environmental release estimation of the Substance were assessed in addition and toxicity to reproduction was identified as additional concern.

The evaluation was conducted by assessing the CSR, original reports for studies included in the registration and the eMSCA's own literature search. The original concern was based on *in silico* (QSAR) information about potential endocrine disrupting properties of the Substance. The *in-silico* information was assessed in detail during substance evaluation. Additionally, *in vitro* data performed by US EPA as part of the Tox 2020 campaign were analysed.

No in-depth analysis of ecotoxicity data was performed as only acute data were available which do not allow assessment of endocrine disrupting properties. Nevertheless, ecotoxicity data are included as supporting information.

In parallel to the substance evaluation, a compliance check for the Substance was conducted by ECHA. A decision issued on 5 November 2015 required the carrying out of an *in vitro* gene mutation study in bacteria and a pre-natal developmental toxicity study in rabbits, oral route.[3]

A meeting between the eMSCA and lead registrant was held in September 2015. Further information and literature references on the Substance were shared by the registrant as a follow-up of the meeting.

A draft decision was issued which required carrying out an EOGRT study (OECD TG 443). Following registrants' comments and consultation of eMSCAs, the draft decision was

referred to the Member State Committee (MSC). The MSC did not reach unanimous agreement on the draft decision, and it was referred to the Commission.

The Commission issued an implementing decision pursuant to Article 52(2) requiring an EOGRT study from registrants of the Substance. The requested study was provided in a dossier update on 13 August 2020 and an original study report to the eMSCA.

7.3. Identity of the substance

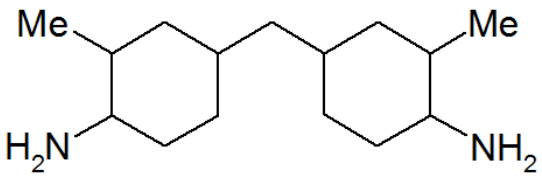
Table 4

SUBSTANCE IDENTITY	
Public name:	2,2'-dimethyl-4,4'-methylenebis(cyclohexylamine)
EC number:	229-962-1
CAS number:	6864-37-5
Index number in Annex VI of the CLP Regulation:	612-110-00-1
Molecular formula:	$C_{15}H_{30}N_2$
Molecular weight:	238.41 g/mol
Synonyms:	DMDC; Cyclohexylamine, 4,4'-methylenebis[2-methyl-; 2,2'-Dimethyl-4,4'-methylenebis(cyclohexylamine); 3,3'-Dimethyl-4,4'-diaminodicyclohexylmethane; 3DCM; 4,4'-Diamino-3,3'-dimethyldicyclohexylmethane; 4,4'-Methylenebis[2-methyl-cyclohexanamine]; 4,4'-Methylenebis[2-methylcyclohexyl-amine]; Bis(3-methyl-4-aminocyclohexyl)methane; Bis(4-amino-3-methylcyclohexyl)methane; Ancamine 2049; Baxxodur EC 331; C 260; Ciba 2976; Dimethyldicykan; EC 331; Epicure 113; Eporesit T 58; Hardener SL; JER 113; JER Cure 113; Laromin C; Laromin C 260; RF 24; Rutapox SL; SIQ-AMIN 1105; T 58.

Type of substance: UVCB

The Substance DMDC is a mixture of a high number of stereoisomers. In the registration, it is classified as a UVCB substance. The very general information about the composition (100% w/w purity) is explained by "a very high number of isomers". The Substance indeed is existent in different isomers. However, the eMSCA notes that the number of possible isomers is not in a range which could not be handled analytically.

Structural formula:



7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20 °C and 101.3 kPa	colourless to yellow liquid
Melting point	–7.1 °C at 101.3 kPa
Boiling point	342°C at 101.3 kPa (Extrapolated value. Substance decomposes above 250 °C)
Vapour pressure	0.0008 hPa at 20 °C (experimental result: carrier gas carry along method)
Water solubility	2.01 g/l at 20 °C (experimental result: OECD TG 105 (Water Solubility))
Partition coefficient n-octanol/water (Log K_{ow})	2.3 at 23 °C and pH 10 1.8 at 23 °C and pH 9 (experimental result: OECD TG 107 (Partition Coefficient (n-octanol / water), Shake Flask Method))
Adsorption coefficient (log K_{oc})	2.6 at 23 °C (pH 7) > 5.63 at 23 °C (pH 9) (read across from experimental result for test material 4,4'-methylenbis[2,6-dimethyl-]-cyclohexanamine; OECD TG 121 Estimation of Koc using HPLC; Klimisch 2; Reuter et al., 2008)
Granulometry	In accordance with column 2 of REACH Annex VII, the particle size distribution does not need to be performed as the Substance is marketed or used in a non-solid or granular form.
Stability in organic solvents and identity of relevant degradation products	In accordance with column 1 of REACH Annex IX, the stability in organic solvents does not need to be tested, because the stability of the Substance is not considered as critical.
Dissociation constant	pKa=10.3 at 25 °C (experimental result: OECD TG 112 (Dissociation Constants in Water))

7.5. Manufacture and uses

7.5.1. Quantities

Table 6

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☒ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

The Substance is mainly used as hardener in epoxy resins (

Table 7). The reactivity of the two amino groups of the Substance with the epoxy component's epoxide groups permits a multiple cross-linkage during hardening, and thus the chemical binding in the cross-linked resins (BUA 1994).

Epoxy resins cross-linked with the Substance are used mainly for coating concrete and other building materials, as raw material for varnishes, and in anti-corrosive paints. These resins can also be employed in shipbuilding and for coating pipelines, as well as in the wet laminating of heavy-duty fibre composite materials (BUA 1994).

Table 7

USES OF DMDC ACCORDING TO THE REGISTRATION (WITH OPPORTUNITY FOR EXPOSURE)	
Identifiers	**Uses where opportunity for exposure arises**
Manufacture (M-1)	Use in batch and other process (synthesis) (PROC 4); **ERC 1 (manufacture)**; spERC: no relevant releases [a].
Formulation (F-2)	Use in batch and other process (synthesis) (PROC 4); Mixing or blending in batch processes for formulation of preparations and articles (multistage and/or significant contact) (PROC 5); **ERC 2 (formulation)**; spERC: marginal release to wastewater [a].
Intermediate in industrial setting (IW-3)	Use in closed, continuous process with occasional controlled exposure (PROC 2); Use in batch and other process (synthesis) (PROC 4); **ERC 6a (use of intermediates)**; spERC: marginal release to wastewater[a]
Use in composite parts of epoxy resins and hardeners (IW-4)	Use in industrial process with possible worker exposure (PROC 2, 4, 5); Industrial spraying (PROC 7); Roller application or brushing (PROC 10); Treatment of articles by dipping and pouring (PROC 13); **ERC 6c (industrial use of monomers for manufacture of thermoplastics)**; spERC: marginal release to wastewater [a].
Professional indoor use of composite materials (PW-5)	Professional handling of substance or preparation with possible worker exposure (PROC 5, 8a, 9); Roller application or brushing (PROC 10); Non-industrial spraying (PROC 11); Treatment of articles by dipping and pouring (PROC 13); Production of preparations or articles by tableting, compression, extrusion, pelletisation (PROC 14); **ERC 8c (wide dispersive indoor use resulting in inclusion into or onto a matrix)**; spERC: similar to wide dispersive use of paints, lacquers and varnishes.
Professional outdoor use of composite materials (PW-6)	Professional handling of substance or preparation with possible worker exposure (PROC 5, 8a, 9); Roller application or brushing (PROC 10); Non-industrial spraying (PROC 11); Treatment of articles by dipping and pouring (PROC 13); Production of preparations or articles by tabletting, compression, extrusion, pelletisation (PROC 14); **ERC 8f (wide dispersive outdoor use resulting in inclusion into or onto a matrix)**; spERC: similar to wide dispersive use of paints, lacquers and varnishes.
Consumer Uses	N/A
Article service life	N/A

[a]) Generally, in the cases of all industrial uses (ES 1-4), the sludge of the local STP is incinerated. Therefore, release of DMDC to (agricultural) soil via sludge is not relevant for these exposure scenarios.

[b]) Release fractions to the environment were provided by the registrant(s).

7.6. Classification and Labelling[4]

7.6.1. Harmonised Classification (Annex VI of CLP)

The Substance has the following entry in Annex VI of Regulation (EC) No 1272/2008:

Table 8

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**		
612-110-00-1	2,2'-dimethyl-4,4'-methylenebis(cyclohexylamine)	229-962-1	6864-37-5	Acute Tox. 4* Acute Tox. 3* Skin Corr. 1A Acute Tox. 3* Aquatic Chronic 2	H302 H311 H314 H331 H411		

The * indicates that manufacturers or importers must apply at least this minimum classification but must classify in a more severe hazard category in the event that further information is available which shows that the hazard(s) meet the criteria for classification in the more severe category (see Annex VI, Section 1.2.1 of the CLP Regulation.

7.6.2. Self-classification

- The self-classification of the registrants contains the following hazard class in addition to the harmonised entry:

  Acute Tox. 2 H330
  STOT RE 2 H373

- The following hazard classes are notified in addition in the ECHA C&L inventory:

  Eye Dam. 1 H318

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1. Abiotic degradation

Based on the chemical structure of the Substance, hydrolysis is not likely to occur under environmental conditions based on a lack of functional groups which may hydrolyse. Therefore, it is concluded that hydrolysis is not an important process in the environmental fate of DMDC. Based on the calculation according to AOPWIN v1.92 (EPI Suite v4.11), DMDC is indirectly photodegradable by reaction with hydroxyl radicals in the atmosphere with a half-life (DT_{50}) of about 3.2 hours taking into account a 24-h day and a mean OH radical concentration of 500,000 radicals per cm^3. The overall OH rate constant was $119.9076 \cdot 10^{-12}$ $cm^3 \cdot molecule^{-1} \cdot sec^{-1}$.

Therefore, it is concluded that, after evaporation or exposure to air, DMDC will be rapidly degraded by indirect photochemical processes. The reliability of the result from the EPI Suite calculation is rated Klimisch 4 (not assignable) by the eMSCA because full documentation is not provided.

[4] ECHA C&L inventory, accessed on 20 August 2020: http://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/cl-inventory/view-notification-summary/23990

7.7.1.2. Estimation of biodegradation

Biodegradation of DMDC has been estimated by calculation with the BIOWIN v4.10 models (EPI Suite v4.11) based on the following calculated parameters: log K_{ow} = 4.10; water solubility = 87.93 mg/L (Table 9).

Table 9

Estimation of biodegradation of DMDC with BIOWIN v4.10			
BIOWIN v4.10 model	**Calculated value**	**Corresponding to**	**ECHA Guide trigger[a]**
Biowin2 (non-linear model)	0.8633	-	< 0.5
Biowin3 (ultimate survey model)	2.7212	weeks – months	< 2.25 – 2.75
Biowin6 (MITI non-linear model)	0.0216	-	< 0.5

a) This value refers to screening criteria for persistence in the ECHA Guidance Chapter R.11, Version 2-2014, Table 4, p. 37; cf. PBT assessment in Section 0.

According to BIOWIN simulations, the degradation of DMDC is not fast. With regard to the persistence criterion, DMDC is a borderline case (cf. PBT assessment in Section 0). The reliability of the result from the EPI Suite calculation is rated 'not assignable' (Klimisch 4) by the eMSCA because full documentation is not provided.

7.7.1.3. Biodegradation

Biodegradation of DMDC was investigated in screening tests: (a) a test on ready biodegradability (OECD TG 301C; key study); (b) two older tests on inherent biodegradability (OECD TG 301B; supporting studies).

(a) The screening test on ready biodegradability (OECD TG 301C; Modified MITI Test (I); NITE Japan, 2001) was rated 'reliable without restriction' (Klimisch 1) by the eMSCA. Under the test conditions (20°C), no biodegradation of DMDC was observed (0% O_2 consumption after 28 d; 3% TOC removal after 28 d).

(b) The two screening tests on inherent biodegradability contained in the registration were rated 'reliable with restrictions' (Klimisch 2). Under the test conditions (20°C), no biodegradation of DMDC was observed (< 1%, resp. 3% degradation of the test substance after 28, resp. 13 d).

According to the biodegradation screening tests, DMDC may be regarded as "not readily and not inherently biodegradable".

Taking into account the use of DMDC in matrices and a very slow release from there, exposure to DMDC in the environment is expected to be low. Therefore, the uncontested persistence of DMDC does not need to be confirmed in higher tier simulation tests. For modelling purposes, the half-life of DMDC may be considered infinite.

7.7.1.4. Conclusions on degradation

Hydrolysis is not considered to be a relevant process of abiotic degradation of DMDC in water. DMDC is subject to rapid degradation by indirect photochemical processes in air, but release to air is not expected.

Simulation of biodegradation with the BIOWIN models show that the degradation of DMDC is not a fast process. According to biodegradation screening tests, DMDC may be considered "not readily and not inherently biodegradable". A water/sediment simulation testing has not been performed.

Therefore, DMDC may be considered as persistent.

7.7.2. Environmental distribution

7.7.2.1. Estimation of physico-chemical parameters for DMDC

Several physico-chemical (PC) parameters are used for the modelling of the distribution of DMDC to environmental compartments. Table 10 shows the results of the estimation of physico-chemical parameters with EPI Suite v4.1 and experimental data from Table 5, where available.

Full documentation of the PC data estimation, in particular that for EPI suite v4.1, is omitted here. The estimations are therefore rated 'not assignable' (Klimisch 4) by the eMSCA.

Table 10

Estimation of physico-chemical parameters for DMDC with EPI Suite v4.1 with some experimental results		
Parameter	**Model / Type**	**Value**
Molecular mass	EPI Suite v4.1 (from SMILES code)	238.42 [Da]
log octanol-water partition coefficient; log K_{ow}	KOWWIN v1.68	4.10
log octanol-water partition coefficient; log K_{ow}	Experimental, OECD TG 107, Shake Flask Method	2.3 at 23 °C and pH 10 1.8 at 23 °C and pH 9
Soil adsorption coefficient K_{oc} / log K_{oc}, at 25 °C	KOCWIN v2.00 – MCI method KOCWIN v2.00 – Kow method	1195; (log) 3.08 [L/kg] 300.5; (log) 2.48 [a] [L/kg]
Water solubility	WSKOW v1.42 WatSol v1.01	87.9 [mg/L] at 25 °C 1667.5 [mg/L]
Water solubility	Experimental, OECD TG 105	2010 [mg/L] at 20 °C
Vapour pressure	Modified Grain method	926 [Pa] at 25 °C
Vapour pressure	Experimental, carrier gas carry along method	0.08 [Pa] at 20 °C
Henry's Law Constant	HENRYWIN v3.20 – Bond method HENRYWIN v3.20 – Group method	$8.11 \cdot 10^{-4}$ [Pa·m³/mol] --- incomplete

[a]) The Registrant's KOCWIN (Kow method) estimation of the adsorption coefficient K_{oc} is 30.35 L/kg, i.e. 1.482 log(L/kg), based on a log K_{ow} of 2.3 (at pH 10).

Referring to the adsorption behaviour, the registrants provided three estimations of the K_{oc}, using EPISuite (MCI method, Kow method) and an estimation normalised to organic carbon for ionisable organic chemicals according to Franco et al. (2008-2010). While the EPISuite estimations (log K_{oc}: 3.08, 1.48) were rated 'not assignable' (Klimisch 4) by the eMSCA, the QSAR by Franco et al. was rated 'reliable with restrictions' (Klimisch 2) by the eMSCA and showed a value of log K_{oc} of 4.49 with hardly any pH-dependency in the range of pH 5-8.

In contrast to that, the read-across from the substance 4,4'-methanediylbis(2,6-dimethylcyclohexanamine) (CAS RN 65962-45-0) (cf. Table 5) showed a dependency of the log K_{oc} on the pH-value in the test according to OECD TG 121. The log K_{oc} increased from 2.6 at pH 7 to over 5.63 at pH 9 (at 23 °C), and therefore higher than for the reference substance DDT (CAS RN 50-29-3). The adsorption coefficient is a key parameter for environmental distribution modelling.

7.7.2.2. Fugacity Level III distribution modelling

When released to the environment, DMDC will be distributed to the environmental compartments in different amounts. Table 11 shows the result of Fugacity Level III distribution modelling using EPI Suite v4.1 with the substance properties calculated within EPI Suite (cf. Table 10) and assuming multiple Level III output with identical initial release rate to the compartments air, water and soil.

The reliability of the result from the EPI Suite calculation is rated 'not assignable' (Klimisch 4), because full documentation is not provided.

Table 11

Distribution of DMDC according to Mackay Level III Fugacity Model (estimation with standard parameters as provided by EPI Suite v4.1)	
Compartment	**mass amount (percent)**
Air	0.036
Water	14.2
Soil	84.8
Sediment	0.94

The results of the distribution modelling and physical-chemical substance properties lead to the conclusion that the major amount of DMDC will adsorb to the soil when it is released to the environment.

7.7.2.3. Distribution in wastewater treatment plants

The dominant route of exposure for DMDC is expected to be wastewater which is treated in sewage treatment plants. Therefore, distribution modelling based on the molecular weight of 238.42 g/mol, the physical-chemical data from Table 5, and the log K_{OC} of 3.08 taken from Table 10, has been conducted with the help of SimpleTreat to estimate the distribution of the Substance in municipal sewage treatment plants (

Table 12). The calculation was done assuming that the Substance is not readily biodegradable (k=0/h) and the reliability of the result was rated Klimisch 4 ('not assignable'), because a full documentation is not provided. In addition, a calculation using the experimental log K_{oc} of 5.63 L/kg (read-across from CAS RN 65962-45-0; cf. Table 5) was provided in the second distribution estimation.

Table 12

Distribution of DMDC in sewage treatment plants (acc. to SimpleTreat 3.0, debugged version; 7 Feb 1997) using different log K_{oc} values		
Summary of distribution	**Percentage**	**Percentage**
log K_{oc} used	**3.08 L/kg**	**5.63 L/kg**
to air	0.0	0.0
to water	98.7	9.5
via primary sludge	0.9	65.5
via surplus sludge	0.4	24.9
degraded	0.0	0.0
Total	*100*	*100*

The results of the calculation lead to the following conclusion for the lower K_{oc}: When the Substance is released into wastewater, it will be predominantly emitted in effluent water. Depending on the use of the WWTP effluent, the Substance may remain in rivers or, in case of irrigation, might be spread to agricultural soil.

In case of the higher K_{oc}, most of the Substance is expected to adsorb to the sewage sludge and only around 10% will go to the effluent water. Since the application of sludge from municipal sewage treatment plants on soil is a common practice in several countries of the EU, an indirect exposure of agricultural soils cannot be excluded.

7.7.3. Bioaccumulation

7.7.3.1. Estimation of bioaccumulation in aquatic organisms

An estimation of bioaccumulation factors (BCF) based on the EPI Suite software (v4.11; US EPA 2012) BCFBAF v3.01 is provided in the registrations. For the calculations, the registrant(s) used the log K_{ow} of 2.3 (measured at 23 °C and pH 10; cf. Table 5). For comparison, BCF values based on the log K_{ow} of 4.1 estimated for DMDC by EPI Suite itself (KOWWIN v.168) are also included in Table 13.

Full documentation of the accumulation estimation with EPI suite v4.1 is omitted here. The estimations are rated Klimisch 4 ('not assignable').

Table 13

Estimation of bioaccumulation in aquatic organisms for DMDC for two different log K_{ow} values			
Source	**Log K_{ow} used**	**BCF (L/kg ww) estimated by regression method [a]**	**BCF (L/kg ww) estimated with Arnot-Gobas method [b]**
BASF SE (2015)	2.3	15.3	21.8
BCFBAF v3.01	4.1	235.6	1036

a) The substance is within the applicability domain of the BCFBAF submodel (BCF; Meylan et al., 1997/1999). b) Upper trophic, incl. biotransformation estimates; the substance is within the applicability domain of the BCFBAF submodel (Arnot & Gobbas, 2003); but as it does appreciably ionise, the estimation may be less accurate.

Measured and estimated log K_{ow} values for DMDC are < 4.5 and estimated BCF values are lower than 2000 L/kg.

7.7.3.2. Bioaccumulation in fish

Bioaccumulation in fish (*Cyprinus carpio*) was tested with a Japanese method equivalent or similar to OECD TG 305 C (NITE Japan, 2002). The test was done under flow-through conditions for an uptake duration of 60 days; the nominal concentration of the test substance DMDC was 0.02 mg/L, thus far below the water solubility of 2010 mg/L. Acute toxicity for fish (LD_{50} 22 mg/L) was 1000-times higher than the test concentration. Therefore, this key study is valid (Klimisch 1, 'reliable without restriction'). The BCF for DMDC in this test was below 60 L/kg. Since it is not apparent from the data provided in the CSR, whether this BCF value refers to whole body or lipid content, the value was normalised to lipid content (ww) with a factor of 7.5 % lipid/wet weight. Hence, the $BCF_{lipid\ normalised}$ for DMDC in fish is determined to be < 800 L/kg_{lipid}.

7.7.3.3. Conclusions on bioaccumulation

For DMDC, estimations of the BCF in aquatic organisms, as well as experimental results with fish indicate a low bioaccumulation potential.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

Acute and long-term toxicity data for fish, *Daphnia* and algae are included as background information for the assessment of the endocrine disrupting properties. They were not assessed in detail by the eMSCA.

7.8.1.1. Fish

Acute toxicity

According to the key study (NITE Japan, 2002) the LC_{50} for fish is 22.4 mg/L obtained in a semi-static OECD TG 203 with *Oryzias latipes.* Results are supported by two additional studies.

Long-term toxicity

No data available.

Results from short-term toxicity tests on fish, *Daphnia* and algae demonstrate that *Daphnia* is the most sensitive taxa. Thus, long-term fish toxicity data are needed with regard to the systemic toxicity of DMDC.

7.8.1.2. Aquatic invertebrates

Acute toxicity

According to the key study (NITE Japan, 2002) with *Daphnia magna*, the EC_{50} for *Daphnia* is 4.57 mg/L in a static OECD TG 202 study. A supporting study without analytics concluded an EC_{50} of 15.2 mg/L.

Long-term toxicity

A GLP-Study in line with OECD TG 211 with *Daphnia magna* (NITE Japan, 2002) resulted in a NOEC of 4.0 mg/L.

7.8.1.3. Algae and aquatic plants

A non-GLP study with *Desmodesmus subspicatus* resulted in an ErC_{50} of > 5.0 mg/L (BASF AG, 1989). The ERC_{10} was 1.25 mg/L.

7.8.2. Terrestrial compartment

Not assessed.

7.8.3. Microbiological activity in sewage treatment systems

Not assessed.

7.8.4. PNEC derivation and other hazard conclusions

Not assessed.

7.8.5. Conclusions for classification and labelling

Following the assessment of the eMSCA, the available data indicates there is no need to adapt the existing harmonised classification of Aquatic Chronic 2 for DMDC.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

No studies on toxicokinetics, metabolism and distribution of DMDC are available. However, based on physico-chemical properties and on acute and repeated dose studies performed by the dermal, inhalation and oral route the registrants conclude that the Substance can be absorbed via the skin, the lung and the intestinal tract. The eMSCA supports these conclusions.

The registrant does not explicitly address absorption percentages via the oral and inhalation uptake routes. For dermal absorption, the registrant suggests a maximal dermal absorption of 0.00257 mg/cm^2/h based on a calculation using IH SkinPerm model. The eMSCA does not concur with these conclusions on dermal absorption. As per guidance

(REACH guidance document on information requirement, Chapter 7c), default values are taken for an estimation of absorption percentage in the absence of experimental data. In the case of DMDC a default of 100 % must be chosen based on physico-chemical properties (molecular weight < 500 and log P_{ow} between –1 and +4). More specifically, the physico-chemical properties of DMDC (molecular weight: 238.41 g/mol, water solubility: 2.01 g/L; log k_{ow} 2.3 at pH 10 and 1.8 at pH 9) point to a high likelihood of dermal absorption. Further, the corrosive nature of the Substance might cause skin damage after contact with skin, increasing the likelihood of skin permeation due to damaged skin. High dermal penetration is further supported by systemic effects seen in an acute dermal toxicity study.

Based on the information available, the eMSCA suggests 100 % absorption for the oral, dermal and inhalation uptake route. The results of the IH SkinPerm model are for the time being not accepted by the eMSCA, as the use of such a calculation model is not foreseen by the ECHA guidelines, as background information on the input parameters for establishing the IH Skin Perm model is lacking and as hitherto available prediction models for skin penetration are considered insufficient (Niemann, 2013).

Thus, the registrants are advised to use 100 % dermal absorption when calculating risks from dermal exposure of workers. In case of risk, either exposure assessment should be revised or an *in vitro* skin penetration study according to OECD TG 428 should be performed to obtain experimental, chemical-specific data.

7.9.2. Acute toxicity and Corrosion/Irritation

Acute toxicity

Not addressed in this dossier (see also section 7.6.2).

Skin and eye irritation

The Substance DMDC is corrosive to the skin and leads to severe skin damage and consequently already has a harmonised classification as Skin Corr. 1A (H314). The available experimental test data are reliable and suitable for the purpose of classification for serious eye damage/eye irritation. Based on the criteria laid down in Regulation (EC) No. 1272/2008, Eye damage 1 (H318) is warranted for eye damaging properties.

7.9.3. Sensitisation

In two non-guideline compliant studies, the Substance did not cause skin sensitisation and the registrants conclude that the substance is not sensitising.

No original study reports were available concerning skin sensitisation. Based on a discussion which had been taken place between registrants and eMSCA, the registrants provided the study by Thorgeirsson (1978) and elaborated why the study results can be considered acceptable (e.g. choice of vehicle, concentrations used). This information was considered acceptable by the eMSCA.

Therefore, DMDC does not need to be considered as a skin sensitiser.

7.9.4. Repeated dose toxicity

Four studies are used in the registration to draw conclusions on the repeated dose toxicity of DMDC: an oral Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test according to OECD TG 422 (BASF, 2019), an oral 90-d oral Repeated Dose Toxicity Study according to OECD TG 408 (BASF, 1990), an oral Extended One Generation Reproductive Toxicity Study according to OECD TG 443 (BASF, 2020) and a 90-d Subchronic Inhalation Toxicity Study according to OECD TG 413 (version 12 May 1981; BASF, 1992). The registrants provided full study reports.

The eMSCA located additional older repeated dose toxicity studies performed with DMDC which were also considered in the substance evaluation to get a broader picture on the inherent toxic properties of DMDC.

Table 14

Oral Combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test (OECD TG 422) (BASF 2019)	
Study Parameters	
Species/strain/sex	Rat, Wistar, males and females
Group size	10/sex/dose
Test substance	2,2'-dimethyl-4,4'-methylenebis (cyclohexylamine)
Purity	100 area-% (complex mixture isomers)
Dose levels	0, 1.5, 5, 15 mg/kg bw/day
Route	Oral
Administration	Gavage
Vehicle	0.5 % CMC (carboxymethylcellulose) in drinking water (stability demonstrated over a period of 7d)
Application volume	10 mL/kg
Exposure	once daily during premating, mating until lactation day 22 in females; during premating, mating until 1 day post mating in males
GLP	yes, apart from metabolome analysis
Study period	2017-2018; report dated 2019

For results on fertility/developmental endpoints see section 7.9.7 – Toxicity for Reproduction.

At 1.5 and 5 mg/kg bw/d no test substance related adverse findings were noted in the respective parental animals and their pups.

At 15 mg/kg bw/d, a variety of test-substance related adverse effects were observed:

- Reduction in food consumption in high (up to 13% below the concurrent control group during the whole premating period) and mid dose (about 8% below control during premating days 7-13) males and in high dose females during lactation (up to 14% below control).
- Decrease in body weight (♂: -5% on mating day 13, up to -8% on mating days 7 and 14 compared to controls; ♀: up to 9% below controls at PND 10 and 13) combined with a reduction in food consumption in both sexes
- Significantly increased total white blood cell (WBC) count ($p \leq 0.05$, 29% deviation compared to controls), lymphocyte count ($p \leq 0.05$, 29.7% deviation compared to controls), monocyte count ($p \leq 0.05$, 62.96% deviation from control) and platelet count ($p \leq 0.01$, 26% deviation compared to controls) in males
- Significantly increased Aspartate-Aminotransferase (AST) activities in males ($p \leq 0.01$, 131.37% deviation from control).
- Increased inorganic phosphate levels in females ($p \leq 0.05$, 45.9% deviation from control). Statistically significantly increased relative liver weight in females (+ 3.018%)
- T4 levels were significantly reduced (-11.9% compared to controls) in males. Mean T4 values were within the historical control range.
- Liver: Vacuolation, hepatocellular (♂: 7/10, minimal in 5, slight in 2; ♀: 10/10, minimal in 7, slight in 2, moderate in 1)
- Brain: Vacuolation, choroid plexus (♂: 10/10, minimal in 3, slight in 7; ♀: 9 out of 10 females, minimal in 5, slight in 4)
- Axillary lymph nodes: Vacuolation, high endothelial venules (♂: 8/10; ♀: 9/10, all minimal)

- Mesenteric lymph node: Vacuolation, high endothelial venules (♂: 7/10, minimal) and 5 out of 10 females (minimal)
- Glandular stomach: Vacuolation, glandular (♂: 7/10; ♀: 8/10, all minimal 1)

Staining of tissues revealed that vacuolation was of phospholipidic nature. Vacuolation was not accompanied by inflammatory or degenerative changes.

Metabolome analysis of plasma revealed significant change of 6.8% metabolites at 15 mg/kg bw/d and significant change of 3.4% metabolites at 5 mg/kg bw/d when compared to untreated controls.

From the results of this study, the study authors derived a NOAEL of 5 mg/kg bw/d for general systemic repeated-dose toxicity.

Table 15

Oral 90-d toxicity study (OECD TG 408) (BASF 1990) **Study Parameters**	
Species/strain/sex	Rat, Wistar, males and females
Group size	10/sex/dose
Test substance	Laromin C 260
PuritPurity	> 99 %
Dose levels	0, 2.5, 12, 60 mg/kg bw/day
Route	Oral
Administration	Gavage
Vehicle	0.5 % CMC (carboxymethylcellulose); stability in vehicle: 15 hr
Application volume	10 mL/kg
Exposure	5 days/week; 3 months
GLP	yes
Study period	18 Feb 1987 – 22 May 1987; report dated 1990

Animals were checked daily for clinical signs; food consumption and body weights were checked weekly. During the application phase, two clinical-chemical and two haematological investigations were performed. After week 8 and 13, blood samples in surviving animals were taken for immunological determinations. Ophthalmoscopic investigations were performed before and at the end of treatment period. After 3 months, surviving animals were killed and investigated histopathologically.

Results

Deaths occurred in the low-dose group (one female after 37 exposures) and mid-dose group (one male, after 47 exposures). Ophthalmology yielded no substance-induced findings. Food intake was considerably reduced at 60 mg/kg bw/d (males from week 4 (by approx. 20 %); females from week 7 (by approx. 12 %)), in males more prominent compared to females. At 12 mg/kg bw/d, a slight reduction in food intake was observed in females but not in males; at the lowest dose, food intake was not reduced. At the highest dose of 60 mg/kg bw/d, body weight was statistically significantly ($p<0.01$ from day 21) lower in males (by approx. 40 % compared to control at study termination) and in females ($p<0.01$ from day 56) (by approx. 20 %) compared to controls at study termination; at 12 mg/kg bw/d, body weight was statistically significantly ($p<0.05$ from day 84) lower in females (by approx. 7 % at study termination).

Haematology

Alanine-Aminotransferase (ALT) and AST were statistically significantly ($p<0.01$) increased in animals of both sexes at the highest dose, AST as well in 12.0 mg/kg bw/d males ($p<0.01$) at the end of the administration period pointing to functional liver disturbances.

Further, the following findings were noted: reduced serum chloride in high dose males and females; statistically significantly decreased creatinine in serum of high dose animals (both sexes); significantly decreased total protein, albumin, globulin and triglycerides in high dose males; significantly increased leucocytes in high dose males and females; increased incidence of lymphocytes with changes in nuclear structure in high dose animals (both sexes). Changes in white blood cells were interpreted as inflammatory responses assumed to occur due to the vacuolar-degenerative changes in heart, liver and kidney. Mean corpuscular value volume (MCV) and mean haemoglobin content (MHC) in erythrocytes were statistically significantly (for MCV: $p<0.01$, for MDC: $p<0.05$) reduced at the highest dose on study day 85.

Urinary findings

A variety of test-substance related changes (dose- and duration-dependent) were observed in urines of treated animals at mid- and high dose, i.e. cell-derived sediments forming circular epithelia without nucleus, increased excretion of erythrocytes and bacteria pointing to kidney damage.

Immunological investigations (IgG determinations) did not reveal any differences between substance-treated and control animals.

The following results were obtained with respect to organ weights (organ weight was determined only for livers, kidneys, testes and adrenal glands) (percentages compared to controls):

- Both sexes: statistically significant increases in relative liver weights in males (34.5 %) and females (48.6 %) at the highest dose and in mid-dose males (6.9 %).
- Both sexes: increased absolute liver weights (22.2 % in males; 16.9 % in females) at high dose.
- Both sexes: increased relative kidney weights at mid-dose (15.6 % in males; 10.1 % in females) and high dose (76.8 % in males; 35.9 % in females).
- Males: increased absolute kidney weight (9.8%) at mid-dose.
- Both sexes: increased absolute (61.8 % in males, 41.3 % in females) and relative (180 % in males; 81.6 % in females) weights of adrenals at the highest dose.
- Males: Decreased absolute (18.6 %) and relative (40.6 %) testes weights in high dose males.

Histopathology

- Liver: microvacuolar degeneration of liver cells in high dose males and females, occasionally accompanied by single cell necrosis.
- Kidneys: vacuolar tubolopathy in mid- and high dose animals of both sexes but more severe in males.
- Heart: damage of myocytes in all male and female high dose animals and in 4 male and 7 female mid-dose animals considered as vacuolar heart muscle degeneration.
- Adrenals: hypertrophy of cortex in high dose animals;
- Thymus and mesenteric lymph nodes: slight necrosis of lymphocytes in the cortex of thymus, slight depletion of lymphocytes in mesenteric lymph nodes.
- Testes: decreased size of testes in 2/10 animals; decreased size of seminal vesicles in 10/10 animals, focal testis atrophy in 4/10 animals, diffuse testis atrophy in 2/10 animals, reduced content in seminal vesicles in 10/10 animals. No information on spermatology.

In female animals, no adverse findings were reported for ovaries or uteri. Oestrous cycle was not investigated. No adverse findings were reported for lungs or brain.

Based on the results of this study, the registrants derive a NOAEL of 2.5 mg/kg bw/d. The eMSCA does not concur with this conclusion. Although the registrants claim that the study has been performed according to OECD TG 408, there was not a daily (as foreseen in OECD TG 408) but a 5 days/week administration schedule.

To account for a 7 day/week administration, the NOAEL of 2.5 mg/kg bw/d is multiplied with 5 and divided by 7, leading to a NOAEL of 1.8 mg/kg bw/d.

Extended One Generation Reproductive Toxicity Study according to OECD TG 443

For more detailed description of the study and results on reproduction/fertility see section 7.9.7 – Toxicity to reproduction.

DMDC was administered to groups of 25 male and 25 female healthy young Wistar rats (test doses: 0, 1.5, 5 and 15 mg/kg bw/d). Parental (P) animals were treated at least for 10 weeks prior to mating to produce a litter (F1 generation), i.e. total treatment time is around 18 weeks (BASF 2020).

Regarding clinical examinations, P males and females of the high- and mid-dose groups (15 and 5 mg/kg bw/d) showed a reduction in food consumption (in high-dose males during premating up to 15% below control, in high-dose females during premating, gestation and lactation up to 11, 13 and 19% below control, respectively and in mid-dose females during lactation up to 14% below control) and water consumption (high-dose males during premating: up to 17% below control, high-dose females during premating, gestation and lactation up to 21, 18 and 23% below control, respectively; in mid-dose males during premating days 28-59 up to 12% below control and in females during premating, gestation and during PND 1 - 2 (up to 18, 16 and 19% below control, respectively).

Mean body weights of the high-dose P males were statistically significantly below the concurrent control values on premating day 28 onwards till the end of the study (up to 22%). Mean body weights were statistically significantly below the concurrent control values for the high-dose P females on premating day 28 onwards till the end of the study (up to 14%) and for the mid-dose P females during gestation (GD 0 and 20: up to 5%) and during lactation (PND 4 – 18: 7%).

Mean body weights were comparable to the concurrent control values in the mid-dose females during the premating period and in the low-dose males and females and mid-dose males during the entire study period.

In P males, body weight change was statistically significantly below the concurrent control values for the high-dose group during premating days 14 - 63, 0 - 63 (up to 79%, 22%, respectively) and study weeks 0 - 2, 3 - 4 and 0 - 4 after the premating period (up to -2.2 g vs. 8.6 g in control). For the mid-dose males, body weight change was decreased during premating days 21 - 28, 35 - 42 and study weeks 0 - 4 after the premating period (about 13%, 17% and 18%, respectively).

Body weight change was statistically significantly below the concurrent control values for the high-dose females during premating days 0 – 7, 28 – 35, 0 – 63, GD 7 – 20 and 0 - 20 (about 15%, 47%, 18%, 23% and 11%, respectively) and for the mid-dose females during GD 14 – 20 and PND 1 - 4 (about 13% and 48%, respectively). Body weight change was comparable to the concurrent control values in the mid-dose females during the premating period and in the low-dose females during the entire study period.

At the high dose tested, terminal body weight was significantly lower in F0 males (-22%) and females (-12%) compared to controls. Histopathology revealed treatment-related findings in following target organs of males and females: brain, oesophagus, eyes with optic nerve, glandular stomach, heart, kidneys, liver, lungs, axillary and mesenteric lymph nodes, pancreas, pituitary gland, and skeletal muscle. Furthermore, the adrenal cortex, left epididymis and seminal vesicles were affected only in male animals.

The main finding in all these organs was a "microvesicular" type of cytoplasmic vacuolation, characterized by the presence of very few to multiple vacuoles, ranging from very fine to small vacuoles (not larger than the nuclei of the cell). Characteristically, if the cytoplasmic vacuolation was abundant, the cells were very clear or pale and increased in size. Vacuoles larger than the cell nuclei were referred by the registrant as "macrovesicular" type of vacuolation which was observed in few organs (brain and seminal vesicles). In 13 out of 16 target organs the vacuolation occurred without additional signs of cytotoxicity. In 3 out of 16 target organs (kidneys, liver and skeletal muscle) the vacuolation was associated

with signs of cytotoxicity (degeneration/regeneration, inflammation and apoptosis/single cell necrosis).

At 5 mg/kg bw/d the incidence and/or grading of the treatment-related vacuolation was generally lower and was not associated with additional signs of cytotoxicity. Organs showing vacuolation in males only were: axillary and mesenteric lymph nodes, heart, left epididymis, pituitary gland and seminal vesicles. Organs with treatment-related vacuolation affecting both males and females were: oesophagus, glandular stomach, kidneys, lungs, pancreas and skeletal muscle.

At 1.5 mg/kg bw/d, no treatment-related findings were noted.

Based on the systemic occurrence of abnormal vacuolation of parenchymal cells in many organs, that in some organs was corroborated by degenerative and inflammatory lesions, observed in both sexes at mid and high doses a NOAEL of 1.5 mg/kg bw/d was derived for systemic repeated-dose toxicity.

This NOAEL was used in the CSR of the lead registrant as starting point for DNEL derivation.

Table 16

90-d inhalation toxicity study (OECD TG 413, version dated 1981) (BASF 1992) **Study Parameters**	
Species/strain/sex	Rat, Wistar, males and females
Group size 10/sex/dose	10/sex/dose
Test substance	Laromin C 260
Purity	> 99 %
Dose levels	0, 2.5, 12, 60 mg/kg bw/day
Route	Oral
Administration	Gavage
Vehicle	0.5 % CMC (carboxymethylcellulose); stability in vehicle: 15 hr
Application volume	10 mL/kg
Exposure	5 days/week; 3 months
GLP	yes
Study period	18 Feb 1987 – 22 May 1987; report dated 1990

Concentrations were selected based on

- an LC_{50} value (4h) of 0.42 mg/L
- results from a 4-week inhalation pilot test using doses of 2, 9 and 27 mg/m³ (only changes in body weight gain observed)
- a 2-week inhalation pilot study using 72 mg/m³ (lethality: 20 %; lung findings)
- findings of sclerodermis in mice after repeated (10 – 17 day) i.p. administration of 6.3 mg/kg bw/d.

Body weight was determined before administration and weekly during the exposure phase. Ophthalmology was performed at begin and at termination (only in high dose and control animals). Animals were checked daily for lethality and clinical signs. Blood was taken before substance administration and on day 87 after administration had started. After study termination, animals were killed, and organs were investigated histopathologically.

Result

Targeted doses were achieved; the percentages of particles with an aerodynamic diameter ≤ 5 µm were 89, 94 and 89 % at 2, 12 and 48 mg/m³ (MMAD about 3.5, 1.5 and 2.8 µm at 2, 12 and 48 µg/L).

In the high-dose group, local signs of irritation were observed in the skin and upper airways (nasal mucosa, slight vacuolisation of olfactory epithelium in 2/10 high-dose males, and in 1/10 high-dose females).

In the high-dose group, slight vacuolation was observed in the nasal cavity along with rarefaction of the olfactory epithelium (mainly in the anterior, craniodorsal parts) in male and female animals.

In the skin of high dose male and female animals, slight broadening of the epidermis along with slight hyperkeratosis was observed in the scapular region.

Body weight: significantly decreased body weight and body weight gain in high-dose males ($p < 0.01$); significantly decreased body weight gain in high-dose females from day 64 ($p < 0.05$).

Lethality: there were no deaths in the control and high-dose group. One female of the 2 mg/m^3 dose and one male animal of the 12 mg/m^3 group died during the exposure phase, deaths were not attributed to substance administration.

Ophthalmology: no difference between control and high dose group.

Clinical chemistry and haematology: significantly increased glutamate-pyruvate-transaminase (GPT (new: ALT)) activities in males at 48 mg/m^3 ($p<0.01$) and 12 mg/m^3 ($p< 0.05$) (values not given) and significantly increased glutamate-oxalacetatetransaminase (GOT (new: AST)) activities in males at 48 mg/m^3 ($p< 0.01$) attributed to liver damage. Significantly decreased haemoglobin ($p<0.05$) and in consequence MCH- and MCHC values in high dose males (values not given); increase of polychromatic erythrocytes in both sexes (attributed to disturbance of haemoglobin metabolism). Decrease of triglycerides in high-dose males (attributed to reduced food intake).

Pathology

Increased absolute lung weights and increased relative weights of liver, kidneys, lungs, testes and adrenal glands in high dose males. Increased relative weights of liver, kidneys and lungs in high dose females.

Histopathology

Slight vacuolisation of olfactory epithelium in high dose males and females. Slight extramedullary haematopoiesis in high dose females; slight tubulonecrosis in kidneys of high dose males. In spleen, haemosiderin was noted in all high dose animals and extramedullary haematopoesis (9/10 high dose females) considered indicative of haemolytic anaemia. A test substance related effect on kidneys was of borderline significance (slight tubular nephrosis in 6/10 high dose males vs. 1/10 male controls; in females 7/10 mid-dose and 9/10 high-dose rats vs. 7/10 control animals) with increased relative kidney weights ($p < 0.01$) and increased urea concentration in females ($p < 0.01$; unchanged in males). In the CSR, a NOAEC of 12 mg/m^3 has been derived from this study.

The eMSCA suggests that, in order to account for daily (i.e. 7 days/week) exposure (exposure in the study was on 5 days per week) the NOAEC should be 8.6 mg/m^3.

Other non-guideline repeated dose studies retrieved from open literature

The following studies were retrieved from the open literature with limited reporting. Effects of DMDC on the brain and skeletal muscle were described in earlier studies, mainly of explorative (non-guideline) nature administering higher dose levels which are briefly described below. These studies are not included in the registration but were considered by the eMSCA during the substance evaluation.

Table 17

Oral 10-day and 28-day studies (Ohshima 1984, Ohshima 1986, Shibata 1990)	
Study Parameters	
Guideline	/
Species/strain	Rat, Fischer
Group size	5 – 10 males/group
Test substance	bis(4-amino-3-methylcyclohexyl)-methane
Purity	unclear
Dose levels	0, 25, 37.5, 50 and 75 mg/kg
Vehicle	olive oil
Route	oral
Administration	not explicitly mentioned, most probably gavage
Exposure	a) 8 times in 10 days (only 50, 75 and 100 mg/kg) b) 17 times in 4 weeks (only 50 and 75 mg/kg) c) 20 – 22 times in 4 weeks
GLP	not mentioned
Study period	unclear, manuscript received for publication 1983

Animals received the test substance as indicated. After the respective treatment periods, clinical-biochemical and histopathological examinations were performed. Reduced body weight gain was observed in animals of high dose groups. An increase of muscle-related parameters (Creatine-Phosphokinase (CPK), Glutamate-Oxalacetate-Transaminase (GOT (new: AST)), creatine), of Monoamine Oxidase (MAO) and alkaline phosphatase was observed. By histopathological examination, dose- and duration-dependent changes were observed in skeletal muscle and in the choroid plexus of the brain. In muscles of high-dose animals, atrophy, degeneration and regeneration of muscle fibres and an increase in fibroblasts were observed.

In group c, haematological tests, clinical-biological tests and histopathological examinations were performed. In groups a and b, clinical-chemical tests (for CPK, MAO, creatine, creatinine) and histopathological examination was performed. Further, the gastrocnemius muscle and the choroid plexus in the brain were examined by electron microscopy and further muscles were examined by light microscopy. A dose-dependent decrease in body weight gain was observed in treated animals. Further, especially animals of higher doses exhibited weakness of the limb muscle. Haematological investigations demonstrated significant decrease of leucocyte counts.

Findings from light microscopy

In muscles of the higher dosage groups, various degrees of atrophy and degenerative or regenerative changes of muscle fibres were observed with increased numbers of interstitial fibroblasts. The gastrocnemius and quadriceps femoris muscles were more severely damaged than the triceps brachii and intercostal muscles.

In the brain choroid plexus, various degrees of swelling and vacuolar degeneration of the epithelial cells were seen, with a dose dependency of the findings: in the mildly damaged cases, swelling of the epithelial cells was slight and only small vacuoles were scattered in the cytoplasm. In severely damaged cases, almost all of the epithelial cells were markedly swollen and their cytoplasm was completely occupied by vacuoles.

Findings from electron microscopy

In the gastrocnemius muscle of treated animals, myofibers were disrupted irregularly, interstitial mesenchymal cells increased in number with various degrees of production of collagen fibres. Further, a number of inclusion bodies with high electron density were

observed within the sarcoplasm of myofibers. In the brain, the vacuolar change of the epithelial cells in the brain choroid plexus as seen by light microscopy was confirmed. The nerve cells in the central nervous system showed no changes.

Histological changes

Histological changes corresponding to scleroderma were not observed, peripheral nerves and other organs (liver, kidney, heart, lung, spleen) showed no remarkable changes by light microscopy.

Table 18

Oral 10-week study (Ohshima 1989) **Study Parameters**	
Guideline	/
Species/strain	Rat, Fischer
Group size	10 males in treatment groups, 5 vehicle controls
Test substance	bis(4-amino-3-methylcyclohexyl)-methane
Purity	unclear
Dose levels	0, 25 and 50 mg/kg
Vehicle	olive oil
Route	Oral
Administration	Gavage
Exposure	50 times in 10 weeks
GLP	unclear
Study period	unclear, publication dated 1989

Animals were treated as indicated above. At the end of the administration period, animals were killed and selected tissues (explicitly mentioned: liver, kidney, heart, lung, brain and skeletal muscle) were prepared for light and electron microscopy. Qualitatively, comparable results to those from studies performed earlier (Ohshima et al., 1984, 1986) were observed. Furthermore, Clara cells (new club cells) of the bronchiolar epithelium in the lungs of treated rats exhibited substance-induced changes. At light microscopy, Clara cells of treated animals were swollen. At electron microscopy, cytoplasm of Clara cells in treated animals showed a marked accumulation of electron-dense inclusion bodies with a lamellar or more complex structure in a dose-related manner. In type I pneumocytes of treated animals, also inclusions were observed. Other pathological changes in the lung were not observed.

Summary on repeated dose toxicity

Two guideline compliant 90-d toxicity studies (an oral study according to OECD TG 408 and an inhalation study performed according to OECD TG 413 (version dated 1981)), an oral Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test according to OECD TG 422 and an Extended One Generation Reproductive toxicity study according to OECD TG 443 was the key information available for evaluating repeated dose toxicity of DMDC.

In the 90-d oral toxicity study performed with DMDC in rats, liver, white and red blood cells, kidneys, adrenal glands and heart were identified as target organs showing vacuolar-degenerative alterations. After adjustment to administration schedule, a NOAEL of 1.8 mg/kg bw/d was derived by the eMSCA from this study.

Across all oral repeated dose studies, including the results from the OECD TG 422 study and the OECD TG 443 study, there was coherent evidence that administration of DMDC at doses of 5 mg/kg bw/d and above induced cytoplasmic vacuolisation of parenchymal cells in a high number of organs. Among others the brain (choroid plexus, pituitary), the

myocard, skeletal muscle and immune organs are affected. At some target sites (kidneys, liver, skeletal muscle-9cellular vacuolisation was accompanied by tissue inflammation, degeneration, and apoptosis/single cell necrosis. In the liver increased activities of transaminases indicative of liver cell dysfunction were seen.

In depth non-guideline studies including electron microscopy with oral uptake up to 4 weeks identified degenerative, atrophic and fibroblastic lesions in skeletal muscle fibres and vacuolar degeneration and swelling of choroid plexus cells in the brain. Swelling of bronchial Clara cells, accumulation of electron-dense material in Clara cells and in type 1-pneumocytes was noted after 10 weeks of oral administration.

The NOAELs for cellular vacuolation were 5 mg/kg bw/d in the OECD TG 422 study and 1.5 mg/kg bw/d in the OECD TG 443 study (treatment time in the OECD TG 422 study was shorter when compared to the OECD TG 443 study).

The registrant suggested to use 1.5 mg/kg bw/d as NOAEL for systemic repeated-dose toxicity and as starting point for DNEL-derivation. The eMSCA agrees to use the lowest dose of 1.5 mg/kg bw/d for DNEL derivation.

Based on adverse effects (haemolytic anaemia, vacuolation of the olfactory epithelium) in an inhalation 90-d toxicity study and after adjustment to administration schedule, a NOAEC of 1.42 mg/m^3 was derived by the eMSCA from the study which can be taken as a starting point for DNEL derivation for the inhalation route.

7.9.5. Mutagenicity

Not addressed in this evaluation.

7.9.6. Carcinogenicity

Not addressed in this evaluation.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

7.9.7.1. Fertility

Based on the initial concern on endocrine disruption and based on the findings on an oral 90-d toxicity study (described in section 7.9.4) an EOGRTS was requested in the substance evaluation decision. The study results with relevance for fertility are described below. The following studies reveal data with relevance to fertility and reproductive performance:

7.9.7.1.1. Oral 90-d toxicity study (OECD TG 408) (see section 7.9.4)

In this study, decreased absolute (18.6 %) and relative (40.6 %) testes weights were observed in high dose (60 mg/kg bw/d) males. Further, in some of the high dose animals, testis atrophy was observed and there was a reduced content in seminal vesicles of high dose animals. Spermatological examinations were not performed in this study. Apart from testis weight, no other reproductive organs were assessed in this study. The study authors attributed testes effects as secondary effects, i.e. as a consequence of severely reduced body weight at this dose level (42 % lower body weight of treated animals compared to controls at study termination). In the 90-d inhalation study on the other hand, increased relative testis weight was observed in males of the highest concentration (48 mg/m^3). In the same studies, no adverse findings were reported for ovaries or uteri. Oestrous cyclicity and apart from histopathology in high dose and control animals, specific endpoints for (anti)androgenic, (anti)oestrogenic or thyroidal modes of actions (thyroid hormone and TSH levels) have not been addressed in these studies.

7.9.7.1.2. Combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test (OECD TG 422) (see section 7.9.4)

The study was performed as a range-finder study for the requested EOGRTS.

Male and female animals were mated overnight in a 1:1 ratio for a maximum of two weeks.

A vaginal smear was prepared after each mating and examined for the presence of sperm. If sperm was detected, pairing of the animals was discontinued. Male and female reproduction data (mating and fertility indices) and female delivery data (gestation and life birth indices, post-implantation loss) were determined.

Oestrous cycle data were evaluated for F0 generation females over a two-week period prior to mating until evidence of mating occurred. Moreover, the oestrous stage of each female was determined on the day of scheduled sacrifice. Anogenital distance measurements were conducted in a blind randomized fashion, using a measuring ocular on all live male and female pups on PND 1. On PND 13, pups were examined for the presence or absence of nipple/areola. The numbers of nipple/areola anlagen were counted.

A non-dose dependent effect on areolas/nipples was reported. The number of areolas/nipples per pup (litter means) on PND 13 was significantly higher at the mid dose (2.48, 2.76, 3.35 ($p<0.05$), and 2.76 at 0, 1.5, 5, and 15 mg/kg bw/d) although the incidence of males displaying areolas/nipples was not affected. In the control group, number of areolas/nipples per pup was at the upper range of the historical controls (0 – 2.5). Neither anogenital distance nor anogenital index were affected in any treatment group. Furthermore in F0 animals, no test-item related effects on oestrus cycle, corpora lutea, spermatogenesis and reproductive performance were observed up to the highest dose of 15 mg/kg bw/d.

Therefore, a NOAEL of 15 mg/kg bw/d was derived from that study for reproductive performance and fertility.

7.9.7.1.3. Extended One-Generation Reproduction Toxicity study (OECD TG 443)

The test substance was administered to groups of 25 male and 25 female healthy young Wistar rats as an aqueous preparation by oral gavage at dosages of 0 (control), 1.5, 5 and 15 mg/kg bw/d (BASF, 2020). Parental (P) animals were treated at least for 10 weeks prior to mating to produce a litter (F1 generation). Pups of the F1 litter (F1 rearing animals) were selected and assigned to 5 different cohorts (1A, 1B, 2A, 2B and 3) which were subjected to specific post-weaning examinations. The study was terminated with the terminal sacrifice of the F1 rearing animals of cohort 1A. In-life and terminal observations were performed as indicated in OECD TG 443. In particular, a detailed clinical observation (DCO) was performed in all P animals and F1 animals in cohorts 1A, 1B, 2A and 3 at weekly intervals. Oestrous cycle data were evaluated for P females over a three-week period prior to mating until evidence of mating occurred. In all cohort 1A females, vaginal smears were collected after vaginal opening until the first cornified smear (oestrous) was recorded.

The oestrous cycle also was evaluated in cohort 1A and 1B females for 2 weeks around PND 75. Moreover, the oestrous stage of each female was determined on the day of scheduled sacrifice. An auditory startle response test was carried out in all animals of cohort 2A on PND 24.

A functional observational battery (FOB) was performed in all animals of cohort 2A on PND 69. Motor activity was measured in all animals of cohort 2A on PND 69.

A T-cell dependent antibody response assay was performed with cohort 3 animals.

Table 19

Extended One-Generation Reproduction Toxicity study according to OECD TG 443 (BASF 2020)	
Study Parameters	
Species/strain/sex	Rat, Wistar, males and females
Group size	25/sex/dose
Test substance	2,2’-dimethyl-4,4’-methylenebis (cyclohexylamine)
Purity	100 area-% (complex mixture of isomers)
Dose levels	0 (control), 1.5, 5, 15 mg/kg bw/day
Route	Oral

Administration	Gavage
Vehicle	0.5 % CMC in drinking water (stability demonstrated over a period of 7d)
Control	vehicle only
Application volume	10 mL/kg
Exposure	once daily during premating, mating until lactation day 22 in females; during premating, mating until 1 day post mating in males
GLP	yes
Study period	August 2018 – March 2018; report dated 2020

7.9.7.1.3.1. Results in parental (P) animals

Table 20

EOGRTS Findings in P animals			
Finding/ Parameter	**Dose [mg/kg bw/d]**	**Males**	**Females**
Food consumption	15	Statistically significantly reduced (compared to control) during the premating days 21 - 42, 56 - 69 and 0 - 69 (up to 12%, 15% and 9%, respectively)	Statistically significantly reduced (compared to control) during premating days 28 - 35, 42 - 49; GD 7 - 20 and the entire lactation period (up to 9%, 11%, 13% and 19%, respectively)
	5	/	Up to 14% reduced food consumption during lactation
	1.5	/	/
Water consumption	15	Decreased up to 17% compared to controls during premating	Decreased up to 21, 18 and 23% below controls during premating, gestation and lactation
Mean body weight	15	Statistically significantly below concurrent control values on premating day 28 onwards till the end of the study (up to 22%)	Statistically significantly below concurrent control values on premating day 28 onwards till the end of the study (up to 14%).
	5	No test substance-related adverse findings	Statistically significantly below concurrent control values during gestation (GD 0 and 20: up to 5%) and during lactation (PND 4 – 18: 7%)
	1.5	No test substance-related adverse findings	No test substance-related adverse findings
Body weight change	15	Statistically significantly below concurrent control values during premating days 14 - 63, 0 - 63 (up to 79%, 22%, respectively) and study weeks 0 - 2, 3 - 4 and 0 - 4 after the premating period (up to -2.2 g vs. 8.6 g in control)	Statistically significantly below concurrent control values during premating days 0 – 7, 28 – 35, 0 – 63, GD 7 – 20 and 0 - 20 (about 15%, 47%, 18%, 23% and 11%, respectively)
	5	Decreased during premating days 21 - 28, 35 - 42 and study weeks 0 - 4 after the premating period (about 13%, 17% and 18%, respectively).	Statistically significantly below concurrent control values for the mid-dose females during GD 14 – 20 and PND 1 - 4 (about 13% and 48%, respectively).
	1.5	Decrease during premating days 7 - 14 (about 11%)	No test substance-related adverse findings

Pathology	5	Axillary lymph nodes: minimal vacuolation of high endothelial venules in 17 of 20 animals; Oesophagus, skeletal muscle: minimal vacuolation in 19 of 20 animals Glandular stomach: minimal vacuolation in 3 of 20 animals Heart: minimal multifocal vacuolation in 2 of 20 animals Kidneys: minimal tubular vacuolation in 7 of 20 animals and minimal tubular degeneration/regeneration in 1 of 20 animals Left epididymis: minimal epithelial vacuolation in 2 of 20 animals Liver: minimal bile duct vacuolation in 1 of 20 animals Lungs: minimal to slight vacuolation of bronchial/bronchiolar epithelium in 19 of 20 animals; minimal vacuolation in bronchial associated lymphoid tissue (high endothelial venules) in 1 of 20 animals Mesenteric lymph nodes: minimal vacuolation of high endothelial venules in 4 of 20 animals; minimal to slight aggregation of macrophages in 6 of 20 animals. Pancreas: minimal to slight acinar vacuolation and minimal ductal vacuolation in 17 of 20 animals Pituitary gland: minimal diffuse vacuolation in 4 of 22 animals Seminal vesicles: minimal to slight epithelial vacuolation in 10 of 20 animals Skeletal muscle: minimal fiber vacuolation in 6 of 20 animals	Oesophagus, skeletal muscle: minimal to slight vacuolation in 18 of 20 animals Glandular stomach: minimal vacuolation in 2 of 20 animals Kidneys: minimal tubular vacuolation in 1 of 20 animals Lungs: minimal vacuolation of bronchial/bronchiolar epithelium in 19 of 20 animals Pancreas: minimal acinar vacuolation in 1 of 20 animals and minimal ductal vacuolation in 5 of 20 animals Skeletal muscle: minimal fiber vacuolation in 6 of 20 animals
	1.5	No test substance-related adverse findings	No test substance-related adverse findings

In female P-animals the mean oestrous cycle duration was comparable amongst the groups. The female mating index calculated after the mating period for F1 litter ranged between 92% and 100% without showing a dose-response.

The mean duration until copulation was detected (GD 0) varied between 2.3 and 2.8 days without any relation to dose levels. The fertility index ranged between 96% and 100% without showing any relation to dosing.

The mean duration of gestation was comparable in all test groups (i.e. between 21.8 and 22.0 days). The gestation index was 100% in in all test groups. The mean number of implantation sites was statistically significantly below the concurrent control values in the high-dose group (12.3 / 12.1 / 11.2 and 10.3 ($p \leq 0.01$) implants/dam at 0, 1.5, 5 and 15 mg/kg bw/d, respectively). The mean value of the high-dose group was outside the historical control range (11.1 – 15.3).

There were no indications for test substance-induced intrauterine embryo-/foeto-lethality since the post-implantation loss did not show any statistically significant differences between the groups (0.6 / 0.6 / 1.1 and 0.8 at 0, 1.5, 5 and 15 mg/kg bw/d, respectively).

The mean number of F1 pups delivered per dam (average litter size) was statistically significantly below the concurrent control values in the mid- and high-dose groups (11.7 / 11.5 / 10.1 ($p \leq 0.01$) and 9.5 ($p \leq 0.01$) pups/dam, respectively at 0, 1.5, 5 and 15 mg/kg bw/d). Both mean values of the high and mid-dose was outside the historical control range being 10.3-14.9).

The rate of liveborn pups was not affected by the test substance, as indicated by live birth indices of 99% / 99% / 100% and 100% respectively at 0, 1.5, 5 and 15 mg/kg bw/d and the number of stillborn pups was comparable between the groups.

Mean body weights of the high-dose male and female F1 pups and both sexes combined were statistically significantly below the concurrent control values during PND 7-21 (up to 11%, 10% and 10%, respectively). Mean body weight change of high-dose male and female pups and both sexes combined was statistically significantly below the concurrent control values during the entire lactation period (up to 16%, 14% and 15%, respectively). No statistically significant treatment-related differences were observed in F1 animals with respect to vaginal opening in females and preputial separation in males.

7.9.7.1.3.2. Findings in F1 animals

(i) Cohorts 1A and 1B (assessment of reproductive and developmental endpoints)

The incidence of pups showing retention of areolas/nipples was dose-dependently and significantly increased in F1 male pups at PND 13 in the mid and high dose group (83.8%, 85.7%, 89.6% ($p<0.05$), and 95.3% ($p<0.01$) at 0, 1.5, 5, and 15 mg/kg bw/d). Both, the mid and high dose incidences, were outside of the historical control range. Although the number of areolas/nipples per pup was counted, no (litter-) mean values per treatment group were reported and no statistical analysis was performed by the study authors. Therefore, the eMSCA calculated the (litter-) mean numbers of areolas/nipples per pup und performed a statistical analysis. The results show a significant increase in areolas/nipple numbers in the high dose (2.5, 2.6, 2.8, and 3.4 ($p<0.01$) at 0, 1.5, 5, and 15 mg/kg bw/d). Upon re-examination on PND 20, no nipples/areolae were detected in any male pups of all test groups. There were no further substance-related effects on reproductive toxicity and fertility in males.

Findings from clinical and pathological investigations performed in cohort 1A are summarised in Table 22. Apart from effects on body weight and some clinical-chemical parameters, main observation was the occurrence of vacuolation in a variety of organs at the high and the mid dose tested. No alterations in the absolute and relative lymphocyte subpopulation cell counts in the spleen tissue (B-, T-lymphocytes, CD4-, CD8-T-lymphocytes and natural killer (NK) cells) were observed in the F1 generation at PND 90 in both sexes.

Table 21

Findings from clinical examinations, clinical pathology and pathology in F1 animals (cohort 1A)			
Finding/ Parameter	**Dose [mg/kg bw/d]**	**Males**	**Females**
Clinical examinations	15	Decreased body weights during study days 42-56 (up to 11 % below control) Decreased body weight change during major parts of the study period (up to 49% below control)	Decreased body weights during study days 49-56 (up to 6 % below control) Decreased body weight change during study days 0-56 (about 8% below control)
	5	No test substance-related adverse findings	No test substance-related adverse findings

	1.5	No test-substance-related adverse findings	No test-substance-related adverse findings
Clinical Chemistry	15	Significantly (p≤0.05) decreased eosinophils (deviation from control: -31.9%); Significantly (p≤0.01) increased AST activities (deviation from control: 115.62%) Significantly decreased TSH levels at PND 90 (deviation from control: -31.75%); within historical control data (HCD)	Increased monocytes (deviation from control: 51.1%, not statistically significant) Significantly (p≤0.01) increased AST activities (deviation from control: 44.27 %)
	5	No test substance-related adverse findings	No test substance-related adverse findings
	1.5	No test-substance-related adverse findings Significantly decreased TSH levels at PND 90 (deviation from control: -36.64 %); within HCD	No test-substance-related adverse findings Significantly increased TSH levels at PND 22 (deviation from control: 29.24 %); within HCD
Pathology	15	Adrenal Cortex: minimal to slight vacuolation of zona fasciculate in 14 of 20 animals Axillary lymph nodes: minimal to slight vacuolation of high endothelial venules in all animals Brain: slight to moderate vacuolation of choroid plexus in all animals Oesophagus: minimal to slight vacuolation of skeletal muscle in all animals Eyes with optic nerve: minimal vacuolation of retinal pigmented epithelium in 10 of 20 animals Glandular stomach: minimal to slight glandular vacuolation in 13 of 20 animals Heart: minimal to slight multifocal vacuolation in 11 of 20 animals Kidneys: minimal to slight tubular vacuolation in 15 of 20 animals; tubular degeneration/regeneration in 3 of 20 animals Left epididymis: minimal epithelial vacuolation in 12 of 20 animals Liver: minimal to slight centrilobular vacuolation in 19 of 20 animals; minimal bile duct vacuolation in all animals Lungs: minimal to slight vacuolation of bronchial/bronchiolar epithelium in all animals; minimal vacuolation of high endothelial venules in bronchial associated lymph tissue in 17 of 20 animals	Axillary lymph nodes: minimal to moderate vacuolation of high endothelial venules in 19 of 20 animals Brain: minimal to slight vacuolation of choroid plexus in 17 of animals Oesophagus: minimal to slight vacuolation of skeletal muscle in 18 of 20 animals Eyes with optic nerve: minimal vacuolation of retinal pigmented epithelium in 7 of 20 animals Glandular stomach: minimal to slight glandular vacuolation in 16 of 20 animals Heart: minimal multifocal vacuolation in 13 of 20 animals Kidneys: minimal to slight tubular vacuolation in 9 of 20 animals Liver: minimal to moderate centrilobular vacuolation in 14 of 20 animals; minimal multifocal inflammation in 2 of 20 animals; minimal to slight bile duct vacuolation in 17 of 20 animals Lungs: minimal to slight vacuolation of bronchial/bronchiolar epithelium in 19 of 20 animals; minimal vacuolation of high endothelial venules in bronchial associated lymph tissue in 15 of 20 animals Mesenteric lymph node:

		Mesenteric lymph node: minimal vacuolation of high endothelial venules in 15 of 20 animals Pancreas: minimal to moderate acinar vacuolation in all animals; minimal to slight ductal vacuolation in all animals Pituitary gland: minimal to slight diffuse vacuolation in 14 of 20 animals Seminal vesicle: minimal to moderate epithelial vacuolation in all males Skeletal muscle: minimal fiber vacuolation in 10 of 20 animals; minimal to slight degeneration/regeneration in 17 of 20 animals	minimal vacuolation of high endothelial venules in 14 of 20 animals Pancreas: minimal to moderate acinar vacuolation in 19 of 20 animals; minimal to slight ductal vacuolation in 19 of 20 animals Pituitary gland: minimal to slight diffuse vacuolation in 13 of 20 animals Skeletal muscle: minimal fiber vacuolation in 3 of 20 animals; minimal to degeneration/regeneration in 5 of 20 animals
	5	Axillary lymph nodes: minimal to slight vacuolation of high endothelial venules in 15 of 20 animals Esophagus: minimal vacuolation of skeletal muscle in 18 of 20 animals Eyes with optic nerve: minimal vacuolation of retinal pigmented epithelium in 2 of 20 animals Heart: Minimal multifocal vacuolation in 3 of 20 animals Kidneys: minimal tubular vacuolation in 1 of 20 animals Lungs: minimal vacuolation of bronchial/bronchiolar epithelium in 3 of 20 animals; minimal vacuolation of high endothelial venules in bronchial associated lymphoid tissue in 2 of 20 animals Mesenteric lymph node: minimal vacuolation of high endothelial venules in 5 of 20 animals Pancreas: minimal acinar vacuolation in 5 of 20 animals; minimal ductal vacuolation in 17 of 20 animals. Seminal vesicles: minimal epithelial vacuolation in 15 of 20 animals	Axillary lymph nodes: minimal vacuolation of high endothelial venules in 1 of 20 animals Esophagus: minimal to slight vacuolation of skeletal muscle in 19 of 20 animals Kidneys: minimal tubular vacuolation in 1 of 20 animals Pancreas: minimal ductal vacuolation in 11 of 20 animals
	1.5	No test-substance-related adverse findings	No test-substance-related adverse findings

In cohort 1B, the following clinical findings were reported at the highest dose tested (15 mg/kg bw/d):

- Decreased water consumption in males during study days 35 - 52 (up to 18% below control) and in females during study days 35 - 38 (up to 16% below control)
- Decreased food consumption in males during study days 42 - 49 (about 12% below control)

- Decreased body weights in males during study days 14 - 49 (up to 12% below control) and in females during study days 35 - 49 (up to 6% below control)
- Decreased body weight change in males during major parts of the study period (up to 36% below control) and in females during study days 7 - 14 (about 10% below control)

No test-substance-related pathological findings were reported.

At the mid dose of 5 mg/kg bw/d decreased body weights were observed in males during study days 42 - 49 (up to 6% below control) and body weight change was decreased up to 13% below control during several parts of the study period.

At the lowest dose tested (1.5 mg/kg bw/d), no test-substance-related adverse finding were reported.

(ii) Findings in Cohorts 2A and 2B (assessment of impact of the administered compound on the developing nervous system)

Significant findings in the DNT cohort 2A included increased rearing in both sexes combined (22% above the control) and an increase in the amplitude and latency of the auditory startle response (28.5% and 28% respectively) in PND 24 males at the high dose. A finding of a 3% decrease in brain weight in PND 22 high dose males was as well reported.

No adverse clinical signs were observed in Cohorts 2A and 2B and no clear-dose-response findings were demonstrated in the locomotor-activity test in Cohort 2A at PND 69. There were treatment-related effects on neuropathology at the high and mid dose. The study authors interpreted neuropathological observations as a direct effect and not a distinctive neurodevelopmental effect.

Table 22

Findings in Cohorts 2A and 2B			
Finding/ Parameter	**Dose [mg/kg bw/d] and Cohort**	**Males**	**Females**
Clinical examination	15, 2A	Decreased food consumption during study days 35-42 (about 10% below control); Decreased body weight change during study days 14-21 and 0-42 (about 11% below control) Decreased terminal body weights	Decreased body weight on study day 21 und during study days 35-42 (up to 8 % below control) Decreased body weight change during study days 0-42 (about 11% below control) Decreased terminal body weights
	15, 2B	Decreased terminal body weights	Decreased terminal body weights
Neuropathology (cohort 2A) Clinical Pathology/Pathology (cohort 2B)	15, 2A	Minimal vacuolation of the retinal pigment epithelium of the eyes in 7 of 10 animals Droplets in the perikaryon of cervical ganglia in all animals (graded minimal to slight) Droplets in the perikaryon of lumbar ganglia in all animals (graded minimal to slight) Minimal vacuolation in trigeminus ganglia in all animals Up to moderate degeneration of muscle fibers of the gastrocnemius muscle in 9 of 10	Minimal vacuolation of the retinal pigment epithelium of the eyes in 6 of 10 animals Droplets in the perikaryon of cervical ganglia in all animals (graded minimal) Droplets in the perikaryon of lumbar ganglia in all animals (graded minimal) Minimal vacuolation in trigeminus ganglia in all animals Up to moderate degeneration of muscle fibers of the gastrocnemius muscle in 9 of 10

		animals Minimal vacuolation of muscle fibers of the gastrocnemius muscle in 9 of 10 animals Minimal vacuolation of the pituitary gland in 1 of 10 animals	animals Minimal vacuolation of muscle fibers of the gastrocnemius muscle in 3 of 10 animals Minimal vacuolation of the choroid plexus in 2 of 10 animals Minimal vacuolation of the pituitary gland in 1 of 10 animals
	15, 2B	No test-substance related adverse findings	No test-substance related adverse findings
Clinical Examination	5, 2A	No test-substance related adverse findings	No test-substance related adverse findings
	5, 2B	Nothing mentioned in CSR	Nothing mentioned in CSR
Neuropathology	5, 2A	Minimal vacuolation of the retinal pigment epithelium of the eyes in 3/10 animals	Minimal vacuolation of the retinal pigment epithelium of the eyes in 1/10 animals
	5, 2B	No test-substance related adverse findings	No test-substance related adverse findings
Clinical examination/ pathology/neuropathology	1.5, 2A	No test-substance related adverse findings	No test-substance related adverse findings
	1.5, 2B	No test-substance related adverse findings	No test-substance related adverse findings

(iii) Findings in Cohort 3 (assessment of impact of the administered compound on the developing immune system)

The evaluation of the effect of the compound on the developing immune system was not included in the substance evaluation decision but was nevertheless performed.

Compared to study controls, a significantly lower anti-SRBC IgM antibody titer was detected in a T-cell dependent antibody response assay in all dose groups in females. Based on median values, anti-SRBC IgM antibodies in females were reduced by -40.8% ($p<0.01$), –44.5% ($p<0.05$), and –62.1% ($p<0.01$) at 1.5, 5, and 15 mg/kg bw/d, respectively compared to the control (positive control of 4.5 mg/kg bw/d cyclophosphamide: –88.4% ($p<0.01$)). A similar trend for lower values (not significant) was apparent in males (–13.7%, –16.5%, –28.0% at 1.5, 5, and 15 mg/kg bw/d, respectively compared to the control; positive control: –83.2% ($p<0.01$)). The study authors attributed the findings in female animals to unusual high values in study controls exceeding that of historical controls (median 61949 U/mL compared to a historical control range of 6652-38297 U/mL) while the titers of the treatment groups were within the control range. However, it should be noted that also the positive control group was within the historical control range (median 7167 U/mL compared to a historical control range of 6652-38297 U/mL). For males, information on historical controls was not provided.

Therefore, the conclusion of the study authors suggesting that these findings are incidental and not treatment related are not supported by the eMSCA.

At the highest dose tested (15 mg/kg bw/d), the following observations were made:

- Decreased water consumption in females during study days 0 - 11 (up to 15% below control)
- Decreased body weights in females during study day 21 - 28 (up to 9% below control)
- Decreased body weight change in females during study days 0 - 28 (about 10% below control)

There were no test-substance-related adverse clinical/pathological finding, and no test-substance-related findings were reported at the lower doses.

7.9.7.1.3.3. Summary

At the high- and mid-dose tested (15 and 5 mg/kg bw/d) the test substance caused reduction in food and water consumption throughout the entire study segments, and most likely this has contributed to the observed decrease in body weight change and terminal body weight in both sexes of P and F1 animals compared to control values. The reduction of food consumption has been observed in earlier studies, in which "severe gastrointestinal intolerance" was reported without further explanation. Histopathology revealed treatment-related findings in a variety of organs in male and female P and F1 animals at the highest and mid-dose tested whereby incidence/grading was lower at 5 mg/kg bw/d. The main finding in all these organs was a "microvesicular" type of cytoplasmic vacuolation, characterized by the presence of very few to multiple vacuoles, ranging from very fine to small vacuoles. In some organs (kidneys, liver and skeletal muscle) the vacuolation was associated with signs of cytotoxicity (degeneration/regeneration, inflammation and apoptosis/ single cell necrosis). Such observations were not made at the lowest dose of 1.5 mg/kg bw/d.
Therefore, for **general systemic toxicity a NOAEL of 1.5 md/kg bw/d** was suggested by the study authors.

In P animals, a lower number of implantation sites and consequently a decreased number of F1 pups was observed at 5 and 15 mg/kg bw/d. The reduction of implantation sites reached statistical significance at the highest dose ($p \leq 0.01$). Secondary to that finding, the mean number of F1 pups delivered per dam was affected (9.5 at the highest dose versus 11.7 pups/dam in control). In the mid-dose group, the mean number of implantation sites was slightly decreased (without statistical significance) and within the range of the historical control data. However, the mean value of delivered pups per dam (10.1 pups/dam) was outside the historical control range showing statistical significance. Due to the dose dependency, the study authors considered the findings in the high- and mid-dose groups as treatment-related and adverse.
Therefore, a **NOAEL of 1.5 mg/kg bw/d was suggested by the study authors for fertility and reproductive performance** of the P generation. The lower number of implantation sites may have contributed to lower bw gain and terminal body weight at high (and mid) dose.

For **developmental toxicity, a NOAEL of 5 mg/kg bw/d was suggested by the study authors** based on the decrease in pup body weight during lactation at the high-dose level of 15 mg/kg bw/d.

For **developmental neurotoxicity in F1 animals, a NOAEL of 15 mg/kg bw/d was suggested by the study authors**. As the neuropathological effects observed at the high dose tested were considered indicative of a direct neurotoxic effect (also observed in repeated dose toxicity studies), the study authors concluded that there were no specific effects on neurodevelopment. The eMSCA concurs with the conclusion that the neuropathological findings should not be considered as a specific neurodevelopmental effect. However, for some findings in the neuro-behavioural testing, a developmental aetiology cannot be excluded.

The eMSCA disagrees with the NOAEL for developmental toxicity as proposed by the study authors and suggests a LOAEL of 1.5 mg/kg bw/d based on the reduced anti-SRBC IgM antibody titers in F1 females of cohort 3.

7.9.7.2. Developmental toxicity

The endpoint developmental toxicity has been addressed within the process of compliance check. As only a developmental toxicity study in a first species was available when compliance check was performed, the request for a developmental toxicity study in a second species was issued by ECHA in 2015.[5]

[5] ECHA CCH Decision number CCH-D-2114309968-35-01/F from 5 November 2015: https://echa.europa.eu/documents/10162/3e228696-1b70-bec3-8840-987282a9aa58

In order to perform that study, the registrants performed a range-finder study in non-pregnant rabbits and a further tolerance study using pregnant rabbits. The full study reports have been made available for the eMSCA.

7.9.7.2.1. Prenatal developmental toxicity in rats

The study was performed according to OECD TG 414 draft version of June 2000 (BASF, 2019). Doses of 5, 15 and 45 mg/kg bw/d were selected based on a preceding range-finder study. Groups of 25 pregnant Sprague-Dawley rats received DMDC once daily by gavage from day 6 to day 19 post-coitum; control animals received vehicle alone (0.5 % CMC). Clear maternal toxicity was observed at the high dose level of 45 mg/kg bw/day especially with regards to corrected body weight gain (-44%) and macroscopic findings (liver) of the dams. A reduction in food consumption by 7% compared to controls during the period of treatment was concomitantly reported. At the mid dose (15 mg/kg bw/day) maternal toxicity was less pronounced. There were no substance related effects with respect to gestational parameters. The sex distribution was similar in the control and treatment groups. No abnormal external or soft tissue findings in foetuses were noted at all doses. A slight but significant retardation of ossification of the skull bones occurred only at the high dose.

The NOAEL for maternal toxicity was 5 mg/kg bw/day whereas for developmental toxicity/teratogenicity, a NOAEL of 45 mg/kg bw/day was derived (high dose tested) (OECD, 2001; CIT, 2001). Thus, from the results of this developmental toxicity study in rats no indications for malformations and developmental toxic effects or endocrine-disrupting properties of DMDC could be observed. However, parameters indicative of endocrine-disrupting properties were not explicitly addressed in this study.

7.9.7.2.2. Prenatal developmental toxicity in rabbits

Dose levels were selected based on a range-finder study in non-pregnant rabbits and a tolerance study in pregnant rabbits. The test substance was administered by oral gavage at 0, 1, 3 and 9 mg/kg bw/d to pregnant White New Zealand Rabbits from GD 6 to GD 28. Control animals received vehicle only.

Food consumption and body weight (BW) of the animals were recorded regularly throughout the study period. The state of health of the animals was checked each day. On GD 29, all females were sacrificed and assessed by gross pathology (including weight determinations of the unopened uterus and placentas). For each doe, corpora lutea were counted and number and distribution of implantation sites (differentiated between resorptions, live and dead foetuses) were determined. The foetuses were removed from the uterus, sexed, weighed and further investigated for any external, soft tissue and skeletal (inclusive cartilage) findings.

Table 23

Prenatal developmental toxicity in rabbits (OECD TG 414) (BASF 2016)	
Study Parameters	
Species/strain/sex	Rabbit, White New Zealand, female
Group size	25 per dose
Test substance	2,2'-dimethyl-4,4'-methylenebis(cyclohexylamine)
Purity:	
Dose levels	0, 1, 3 and 9 mg/kg bw/d
Route	Oral
Administration	Gavage
Vehicle	0.5 % CMC in drinking water (guarantee statement on stability over testing period)
Application volume	10 mL/kg
Exposure	once daily on gestation day (GD) 6 until GD 28
GLP	Yes
Study period:	July – November 2016
Species/strain/sex	Rabbit, White New Zealand, female

At the highest dose tested (9 mg/kg bw/d) the average food consumption was decreased (19% less compared to controls during GD 6-28, decrease statistically significant between GD 14 and 20). Also body weight and body weight changes were statistically significantly reduced during the treatment period (82% less body weight gain if calculated for the entire treatment period). No substance-related adverse effects were observed in foetuses. At the mid dose (3 mg/kg bw/d), body weight and average body weight change were reduced, the latter being statistically significant between GD 9 and 11. Animals gained 50% less body weight during treatment. Corrected body weights and body weight changes were not significantly different between all test groups. In the high dose group, corrected body weight was 3.3% lower compared to the controls (not significant). No test-substance-related adverse effects were observed in foetuses. At the lowest does (1 mg/kg bw/d), no adverse effects were observed in parental animals and foetuses.

The number of implantation sites was significantly lower in the mid- and high-dose groups (10.9, 10.5, 8.8 ($p<0.05$), and 9.0 ($p<0.05$) at 1, 3, and 9 mg/kg bw/d, respectively compared to the controls). Subsequently, the number of live foetuses (live litter size) was statistically significantly lower in the mid- and high-dose groups (10.2, 9.4, 8.4 ($p<0.05$), and 8.4 ($p<0.05$) at 1, 3, and 9 mg/kg bw/d, respectively compared to the controls). There was a trend for a higher percentage of preimplantation losses (not significant) with increasing dose (1.6%, 4.8%, 9.6%, and 10.1% at 1, 3, and 9 mg/kg bw/d, respectively). Gravid uterus weight was statistically significantly decreased in the mid and high dose (-11.34%, -20.08% ($p<0.01$), -19.12% ($p<0.05$) at 1, 3, 9 mg/kg bw/d, respectively compared to the controls). The study authors attributed the effects in the mid- and high-dose group to the unusually high number of implants and litter size of the concurrent control group. Their values were distinctly above the upper range of the historical control data (implantation sites: 10.9 vs 7.1 – 10.3, litter size: 10.2 vs 6.6 - 9.7). As the mean implant/litter size values of the mid- and high-dose group were close to the mean of the historical control ranges (8.7 and 8.1, respectively) and as no relation to the dose was observed for any of the two apparent effects, they were not considered to be treatment-related by the study authors.

Incidences of individual skeletal variations were well inside the historical control range. Therefore, these minor changes were not considered as treatment-related adverse events by the study authors.

Based on the results of this study, a NOAEL of 1 mg/kg bw/d was derived for maternal toxicity based on reduced food consumption and reduced body weight/body weight gain. Based on the absence of adverse findings on foetuses, a NOAEL of 9 mg/kg bw/d was derived for foetal (developmental) toxicity.

The eMSCA is of the opinion that the statistically significantly lower number of implantation sites and number of pups at the mid and high doses should be considered treatment-dependent and adverse.

In the Combined Repeated Dose Toxicity Study with the Reproduction/developmental Toxicity Screening Test (OECD TG 422) (see also Sections 7.9.7.1 and 7.9.4 above) in Wistar rats, no treatment-related effects on foetuses were reported up to postnatal day (PND) 13 and up to the highest dose tested (15 mg/kg bw/d). Therefore, a NOAEL of 15 mg/kg bw/d was derived from that study for developmental toxicity.

7.9.7.3. Summary on Toxicity for reproduction

Several studies are available addressing reproductive toxicity of DMDC:

* in a OECD TG 422 study, no test-item related effects on oestrus cycle, sperm parameters and reproductive performance were observed up to the high dose of 15 mg/kg bw/d.

* In a OECD TG 443 (2018), a lower number of implantation sites and consequently a decreased number of F1 pups was observed at 5 and 15 mg/kg bw/d. Therefore, a NOAEL of 1.5 mg/kg bw/d was suggested by the study authors for fertility and reproductive performance of the P generation. For developmental toxicity, a NOAEL of 5 mg/kg bw/d was suggested by the study authors based on the decrease in pup body weight during lactation at the high-dose level of 15 mg/kg bw/d.

Based on the findings of reduced anti-SRBC IgM antibody titres in F1 females of cohort 3 in all dose groups, the eMSCA suggests a LOAEL of 1.5 mg/kg bw/d for developmental toxicity. Based on the neuropathology, the eMSCA considers that DMDC does not exert specific neurodevelopmental toxicity (neuropathological findings were observed as well in adult animals), although for some findings in neurobehavioural testing a developmental aetiology cannot be excluded. The neuropathological as well as the neurobehavioural findings in the F1 animals of the OECD TG 443 study are, however, covered by the LOAEL of 1.5 mg/kg bw/d for development.

* In two OECD TG 414- one performed in Sprague-Dawley rats (June 2000) and pme performed in white New Zealand rabbits (2001), no specific adverse effects on development could be observed.

A lower number of implantation sites along with a decreased number of pups was reported in the EOGRTS in Wistar rats at 5 and 15 mg/kg bw/d as well as in the developmental toxicity study (OECD TG 414) performed in rabbits at 3 and 9 mg/kg bw/d, but not in the developmental toxicity study performed in rats.

Therefore, the eMSCA is of the opinion that for both fertility and development, NOAELs/LOAELs, should be derived from the EOGRTS (i.e. a NOAEL of 1.5 mg/kg bw/d for fertility and reproductive performance and a LOAEL of 1.5 mg/kg bw/d for development).

For risk assessment purposes and derivation of DNELs, the (developmental) LOAEL of 1.5 mg/kg bw/d from the EOGRTS performed in rats should be used as the starting point.

7.9.8. Hazard assessment of physico-chemical properties

Not part of the eMSCA's assessment.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Not part of the eMSCA's assessment.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

The eMSCA disagrees with the proposal to classify the Substance as STOT RE 2. Instead, the Substance should be classified as STOT RE 1 as vacuolation was observed in a variety of organs and in different independent repeated dose toxicity studies, which manifested in tissue damage at higher doses, already at 5 mg/kg bw/d.

For risk assessment purposes and DNEL-derivation the LOAEL of 1.5 mg/kg bw/d from the EOGRTS (OECD TG 443) performed in rats should be used as the starting point.

A lower number of implantation sites along with a decreased number of pups was reported in the EOGRT study (OECD TG 443) in Wistar rats at 5 and 15 mg/kg bw/d as well as in the developmental toxicity study (OECD TG 414) performed in White New Zealand rabbits at 3 and 9 mg/kg bw/d, but not in the developmental toxicity study (OECD TG 414) performed in Sprague-Dawley rats. Therefore, based on the available information, the eMSCA considers DMDC as a reproductive toxicant. Consequently, the eMSCA considers a harmonised classification according to CLP either a Repr. 2 or 1B as appropriate.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

7.10.1.1. *In silico* information

The original concern was based on a structural alert for DMDC with regard to oestrogen receptor binding.

According to the OECD QSAR Toolbox (version 3.3 and previous versions) DMDC is a strong oestrogen binding substance based on the oestrogen receptor binding profiler developed

by LMC. The alert is based on the following profile: "MW > 200 and MW ≤ 500 and with a non-impaired NH_2 group attached to 5 or 6 C-atoms ring".

This structural alert originates from a publication by Hamblen *et al*., (2003), which assessed the oestrogen receptor binding profile of aromatic amines. Thus, although the structural alert accounts for both aromatic and non-aromatic amines, it is validated for aromatic amines only.

Regarding DMDC, its potential binding capacity is expected to be reduced due to its high pK_a value (pK_a 10) and thus its high protonation rate, introducing a new ionic interaction potential, at physiological pH. Furthermore, the non-planar structure of the non-aromatic six-membered carbon rings is considered to decrease the binding affinity of DMDC to the oestrogen receptor compared to aromatic analogues.

In summary, although DMDC offers hydrophobic interaction potential in combination with a hydrogen bond donor potential, its structure-based oestrogen receptor binding potential is considered as low due to the missing aromatic rings, a positive charge at physiological pH and a missing rigid structure as observed for natural steroids.

7.10.1.2. *In vitro* information

DMDC is included in the large substance library screened within the US EPA Tox21 program. The substance was screened with respect to its in vitro potency in different cell tests including oestrogen, androgen, and thyroid receptor as well as aromatase assays. This *in vitro* screening reveals only negative or inconclusive results for DMDC regarding diagnostic endpoints for an ED mode of action. Hence, the available *in vitro* data also do not raise a concern for an EAS- (oestrogen-androgen steroidogenesis) and T-mediated endocrine activity of DMDC.

7.10.1.3. *In vivo* information

No long-term ecotoxicity data are available for DMDC. Therefore, no *in vivo* information on a possible endocrine mode of action and correlated adverse effects is available with regards to fish or amphibians. As discussed in section 7.10.2., the provided mammalian data do not show population relevant adverse effects which could be biologically plausibly linked to an endocrine mode of action.

7.10.2. Endocrine disruption - Human health

Owing to the initial concerns about the potential endocrine-disrupting properties of DMDC, the eMSCA prepared a draft decision in accordance with Article 46(1) of Regulation (EC) No 1907/2006 including an information request for an EOGRTS to be conducted in rats via the oral route (test method: EU B.56/OECD TG 443) with the expansion of Cohort 1B to produce the F2 generation to address potential endocrine-disrupting properties. The draft decision was referred to the MSC. The MSC considered that the testis atrophy indicative for a concern on potential endocrine modulating effects observed in the 90-d study is likely a consequence of high general toxicity and not linked to a potential endocrine mode of action. The MSC therefore agreed by consensus that the initial concern about endocrine disruption was insufficient grounds to request for extension of the Cohort 1B to include the F2 generation (see EU C (2018) 2755 final).

Results from the EOGRTS (OECD TG 443) in rats show a decrease in implantation numbers and litter size. Similar findings were reported in the OECD TG 414 study in rabbits. Implantation numbers and litter size are parameters sensitive to but not diagnostic for the EAS modality.

In the EOGRTS (OECD TG 443), the incidence of pups showing retention of areolas/nipples was dose-dependently and significantly increased in F1 male pups at PND 13 in the mid and high dose group. Retention of areolas/nipples in male pups might indicate some anti-androgenic activity of the test substance. However, the increase was weak and showed full recovery upon re-examination after weaning. Since no other EAS sensitive parameters were affected in males and the *in vitro* data are inconclusive, this finding alone does not provide strong evidence for an ED mode of action and is insufficient to consider DMDC as an ED with regards to the EAS modality.

Effects of DMDC were also observed with regard to the thyroid system. In the OECD TG 422 study, T4 levels were significantly reduced (-11.88% compared to controls) in parental (P) males at the high dose. Similarly, in the EOGRTS, T4 levels were reduced in the high dose in P males (-15.43% compared to controls). Furthermore, in F1 female pups on PND 22, TSH was significantly increased in the low dose (29.24% compared to controls), and in F1 males on PND 90, TSH was significantly decreased in the low and high dose group (-36.64% and -31.75%, respectively). Thyroid histology was not affected by treatment, and any changes in thyroid weight could be explained by body weight effects of the test substance. The observed changes in T4 and/or TSH levels might indicate an interaction of DMDC with the thyroid hormone system. However, changes in hormone levels were rather weak. For TSH, there was no apparent dose-response and effect direction, and no correlation between TSH and T4 levels was obvious. In addition, since the *in vitro* data are inconclusive, possible molecular initiating events remain unknown.

In summary, although DMDC induced changes in EATS-sensitive parameters, it remains unknown whether the test substance acts via a specific endocrine mode of action. Furthermore, the observed adverse effects on fertility indicate reproductive toxicity but cannot plausibly be linked to an endocrine mode of action.

Therefore, the Substance is not considered as an ED for human health.

7.10.3. Conclusion on endocrine disrupting properties (combined/ separate)

DMDC was originally considered for substance evaluation based on a structural alert pointing to a possible oestrogenic activity.

The assessment of the available *in silico* information and further structure-activity considerations led the eMSCA to the conclusion that the oestrogen receptor binding potential of DMDC is low.

In-depth analysis of the available *in vitro* data led the eMSCA to the conclusion that there is no strong concern for an endocrine activity of DMDC mediated via oestrogen, androgen and thyroid receptor interaction as well as via inhibition of aromatase. The *in vitro* assays were negative or inconclusive as activity was observed only at concentrations causing cytotoxicity in the assay systems.

In vivo studies in rats show some impacts on EATS sensitive parameters but there is no evidence that DMDC acts via a specific endocrine mode of action. Therefore, DMDC is not considered as an ED for human health.

No studies are available for DMDC to conclude on endocrine specific endpoints in aquatic species. However, considering the available *in silico*, *in vitro* and *in vivo* data from the mammalian assessment, the eMSCA comes to the conclusion that DMDC should not be considered as an endocrine disruptor in the environment and that further testing to follow up on the concern is not necessary.

7.11. PBT and vPvB assessment

7.11.1. Persistence

Degradation of DMDC has been assessed in Section 7.7.1. The outcome and the P-assessment are presented in Table 24.

Table 24

Assessment of persistence for DMDC			
Persistence data	**Degradation values**	**ECHA Guide trigger**	**Conclusion**
Biowin 2 and Biowin 3 [b]	0.8633 and 2.7212	< 0.5 and < 2.25-2.75	Potentially P [a]
Biowin 6 and Biowin 3 [b]	0.0216 and 2.7212	< 0.5 and < 2.25-2.75	Potentially P [a]

Ready biodegradability	not readily biodegradable	< 60% ThOD	Potentially P or vP
Half-life in a water/ sediment simulation test	DT_{50} values not available	> 40 d (fresh water) > 120 d (sediment) [c]	-

[a]) With regard to BIOWIN3, the value for DMDC (2.7212) is a borderline case. The ECHA Guidance Chapter R.11 states on p. 37: *"For substances fulfilling this, but BIOWIN indicates a value between 2.25 and 2.75, more degradation relevant information is generally warranted."*

[b]) Full documentation of the degradation estimation with EPI suite v4.1 is omitted here. The estimations are therefore rated Klimisch 4 (not assignable)

[c]) Cf. persistence criteria according to Annex XIII of the REACH Regulation (other values for the marine environment).

Since DT_{50}-values for DMDC are not available, a definite conclusion on the P-criterion cannot be drawn. Therefore, DMDC needs to be considered as potentially P or vP according to the criteria of REACH Annex XIII.

7.11.2. Bioaccumulation

The bioaccumulation potential of DMDC has been assessed in Section 7.7.3. The outcome and the B-Assessment are presented in Table 25.

Table 25

Assessment of bioaccumulation potential for DMDC			
Bioaccumulation data	**Bioaccumulation values (L/kg)**	**ECHA Guide trigger (B / vB) [a]**	**Conclusion**
max. log K_{ow} (calc.)	4.1	4.5	No bioaccumulation potential
Estimation BASF SE (2015)	15.3 / 21.8 [b]	< 2000 < 5000	Not bioaccumulating
BCFBAF v.301 (log K_{ow} used 4.1)	235.6 / 1036 [b]	< 2000 < 5000	Not bioaccumulating
OECD 305 C (NITE Japan, 2002)	< 800 [c]	< 2000 < 5000	Not bioaccumulating

[a]) In accordance with REACH-Regulation Annex XIII. Trigger values relate to BCF lipid normalised.

[b]) Estimated with BCFBAF v3.01 by regression method / Arnot-Gobas method.

[c]) Value normalised to 7.5 % lipid content for *Cyprinus carpio*.

Based on estimations of the bioaccumulation potential (reliability not assignable) and an experimental result from a fish test, DMDC is considered as not bioaccumulative according to the criteria of REACH Annex XIII (not B/vB).

7.11.3. Toxicity

The ecotoxicity of DMDC to aquatic organisms was investigated in several studies and has been assessed in Section 7.8.1.

Short-term data are available for fish, *Daphnia*, algae, and micro-organisms. The lowest short-term ecotoxicity value is a 48-h EC_{50} for *Daphnia* at 4.6 mg/L (nominal).

There are no long-term toxicity data for fish; fish are not the most sensitive species in acute testing. A long-term toxicity testing of DMDC with *Daphnia* resulted in a 21-d NOEC of 4 mg/L (nominal). For algae, the 72-h NOErC was measured at 0.13 mg/L (geometric mean).

Short-term toxicity of DMDC to aquatic organisms is above 0.01 mg/L, the trigger value for the screening criterion for aquatic toxicity according to REACH Annex XIII. Likewise, the long-term toxicity of DMDC to aquatic organisms is higher than the trigger value of 0.01 mg/L. Hence, DMDC is considered to be non-toxic to aquatic organisms according to the criteria of REACH Annex XIII.

However, DMDC is toxic for human health: Identified target organs are skeletal muscle, myocardium, kidney and liver and reproductive effects have been observed for the substance. The eMSCA considers that a classification of DMDC as STOT RE 1 and reproductive toxicity is warranted. Hence, the substance is considered to fulfil the T-criterion according to REACH Annex XIII.

7.11.4. Conclusion on the PBT properties of DMDC

Compared to the criteria of REACH Annex XIII, DMDC is persistent, not bioaccumulating and not toxic to aquatic organisms. However, DMDC may fulfil the T criterion with respect to human health according to REACH Annex XIII based on the potential for classification as STOT RE and as a reproductive toxicant.

Therefore, DMDC is potentially P/vP, not B, and T. The eMSCA concludes that DMDC cannot currently be regarded as a PBT or vPvP substance.

7.12. Exposure assessment

7.12.1. Human health

7.12.1.1. Worker

Not assessed during this substance evaluation.

7.12.1.2. Consumer

No Consumer uses were indicated by in the registration.

7.12.2. Environment

Based on the exposure scenarios for DMDC (

Table 7), the eMSCA has performed own calculations of the refined compartment-specific PEC values with EUSES v2.1.2. These PEC values are not identical, but more or less in the same range as the PEC values provided by the registrant(s). The evaluating Member State concludes that these PEC values are acceptable for risk characterisation (Section 7.13).

7.12.3. Combined exposure assessment

Not applicable.

7.13. Risk characterisation

Environment:

Risk Characterisation Ratios (RCR) have been determined for all relevant environmental compartments, based on PEC values (cf. Exposure Assessment, Section 7.12.2) and PNEC values provided by the registrant(s). The registrant(s) conclude that all RCR for environmental compartments are below 1. The eMSCA supports this conclusion.

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7.15. Abbreviations

ALT	Alanine-aminotransferase
AR	Androgen Receptor
AST	Aspartate-aminotransferase
CoRAP	Community Rolling Action Plan for Substance Evaluation
CPK	Creatine Phosphokinase
CSR	Chemical Safety Report
DMDC	2,2'-dimethyl-4,4'-methylenebis(cyclohexylamine)
DNEL	Derived No Effect Level
ECHA	European Chemicals Agency
ED	Endocrine disruptor
eMSCA	evaluating Member State Competent Authority
ERE	Oestrogen Responsive Elements
GR	Glucocorticoid Receptor
IC_{50}	half-maximal inhibitory concentration
IgG	Immunoglobulin G
MAO	Monoamine-oxidase
MMAD	Mass Median Aerodynamic Diameter
MCV	Mean Corpuscular Volume
MSC	Member State Committee
NOEC	No Observed Effect Concentration
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PROC	Process Category
P(N)EC	Predicted (No) Effect Concentration
RCR	Risk Characterisation Ratio
PACM	4,4'-methylenebis(cyclohexylamine) (CAS RN 1761-71-3, EC No 217-168-8)
pkA	acid dissociation constant at logarithmic scale
qHTS	quantitative high-throughput screening
QSAR	Quantitative Structure-Activity Relationship
spERC	(specific) Environmental Release Category
STOT RE	Single Target Organ Toxicity Repeated Exposure
T3	Triiodothyronine
TR	Thyroid Receptor
Ww	Wet weight
WWTP	WasteWater Treatment Plant