Committee for Risk Assessment

RAC

Annex 1

Background document

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

Desmedipham (ISO); ethyl 3-phenylcarbamoyloxyphenylcarbamate

EC Number: 237-198-5
CAS Number: 13684-56-5; (125579-95-5); (153703-69-6)

CLH-O-0000001412-86-294/F

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

Adopted

20 September 2019

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

desmedipham (ISO); ethyl 3-phenylcarbamoyloxyphenylcarbamate

EC Number: **237-198-5**

CAS Number: **13684-56-5**

Index Number: **616-113-00-9**

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Version number: 2 **Date: 12.11.2018**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DESMEDIPHAM (ISO); ETHYL 3-PHENYLCARBAMOYLOXYPHENYLCARBAMATE

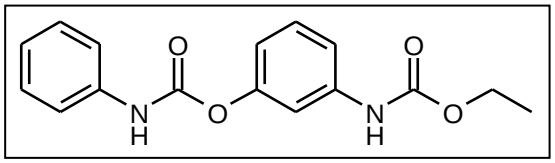
CONTENTS

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DESMEDIPHAM (ISO); ETHYL 3-PHENYLCARBAMOYLOXYPHENYLCARBAMATE

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	ethyl 3-phenylcarbamoyloxycarbanilate
Other names (usual name, trade name, abbreviation)	
ISO common name (if available and appropriate)	desmedipham (ISO)
EC number (if available and appropriate)	237-198-5
EC name (if available and appropriate)	desmedipham
CAS number (if available)	13684-56-5
Other identity code (if available)	477
Molecular formula	$C_{16}H_{16}N_2O_4$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	300.3 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	
Description of the manufacturing process and identity of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	min. 980 g/kg min. 970 g/kg

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
desmedipham, CAS 13684-56-5	97-98%	Aquatic Acute 1, H400 Aquatic Chronic 1, H410	Aquatic Acute 1, H400 Aquatic Chronic 1, H410

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
toluene, CAS 108-88-3	max 2g/kg (0.2%)	Flam.Liq 2, H225 Skin Irrit. 2, H315 Asp- Tox. 1, H304 STOT SE 3, H336 STOT RE 2, H373 Repr. 2, H361d		No
3-aminophenol, CAS 591-27-5	max 1g/kg (0.1%)	Acute Tox. 4*, H302 Acute Tox. 4*, H332 Aquatic Chronic 2, H411		No
aniline, CAS 62-53-3		Acute Tox. 3*, H301 Acute Tox 3*, H311 Acute Tox 3*, H331 Eye Dam. 1, H318 Skin Sens. 1, H317 Muta. 2, H341 Carc. 2, H351 STOT RE 1, H372 Aquatic Acute 1, H400		No

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling

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

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	616-113-00-9	*desmedipham (ISO); ethyl 3-phenylcarbamoyloxyphenylcarbamate*	237-198-5	13684-56-5	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M = 10	
Dossier submitters proposal		desmedipham (ISO); ethyl 3-phenylcarbamoyloxyphenylcarbamate	237-198-5	13684-56-5	**Add** Repr. 2 STOT RE 2 **Retain** Aquatic Acute 1 Aquatic Chronic 1	**Add** H361d H373 (blood) **Retain** H400 H410	**Add** GHS08 **Retain** GHS09 Wng	**Add** H361d H373 (blood) **Retain** H410		**Modify** M = 10 **Add** M = 10	
Resulting Annex VI entry if agreed by RAC and COM		desmedipham (ISO); ethyl 3-phenylcarbamoyloxyphenylcarbamate	237-198-5	13684-56-5	Repr. 2 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H361d H373 (blood) H400 H410	GHS08 GHS09 Wng	H361d H373 (blood) H410		M = 10 M = 10	

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

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

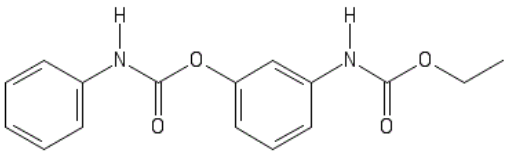
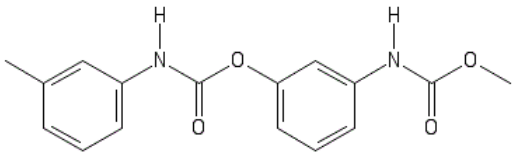
3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The hazard classification of desmedipham according to Dangerous Substances Directive (DSD) 67/548/EEC was first agreed in the September 2000 meeting of the Commission Working Group on the C&L of Dangerous Substances (Pesticides – Environmental effects). Specific concentration limits, corresponding M-factor of 10, were added to the N; R50-53 classification in the June 2003 meeting. The classification N, R50-53 was included in Annex 1 of DSD in the 29th ATP (Commission Directive 2004/73/EC of 29 April 2004). The DSD classification was translated to CLP Classification Aquatic Acute 1, M=10; Aquatic Chronic 1 in Annex VI of CLP.

RAC general comment

Desmedipham is an herbicide from the phenylcarbamate group.

The dossier submitter (DS) used data on a structurally related substance, phenmedipham, as supporting information in the assessment of several effects. According to the DS, the chemical structure, chemical properties, breakdown products and toxicological profiles of desmedipham and phenmedipham are similar. The structures of both substances are shown below.

	
Desmedipham	**Phenmedipham**

As to the metabolic profile, RAC notes that although both substances are converted to aromatic amines and their derivatives, the metabolites are not identical or their relative amounts are different (see CLH report of phenmedipham, p. 10; CLH report of desmedipham, p. 10; summaries of ADME studies in both RARs). RAC further notes several differences between the toxic effects of desmedipham and phenmedipham: (1) although both substances are haematotoxic, desmedipham is more potent; (2) in addition to haematotoxicity, desmedipham affected the thyroid while phenmedipham did not in the available studies; (3) desmedipham, unlike phenmedipham, induced slightly increased incidence of several malformations such as micrognathia and cleft palate in rat prenatal developmental toxicity (PNDT) studies.

Since RAC considers the available information on repeat dose toxicity, carcinogenicity and reproductive toxicity of phenmedipham to be conclusive, RAC does not see a need to include data on phenmedipham in the assessment.

The study numbers in the human health part refer to the respective sections of the RAR (draft Renewal Assessment Report under Regulation (EC) 1107/2009, RMS Finland, December 2017).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

5 IDENTIFIED USES

Desmedipham is a non-systemic contact herbicide. It acts only via the foliage of emerged weeds and therefore does not depend on soil type or humidity; its efficiency depends on temperature and light intensity. Desmedipham controls a wide range of broad-leaved weeds. It acts only via the foliage emerged weeds and inhibits the Hill-reaction. Desmedipham leads to foliar discolouration followed by chlorosis and withering, and finally complete death of weeds.

6 DATA SOURCES

The Renewal Assessment Report (2017) under Regulation (EC) 1107/2009 was used as the main data source for drafting the CLH report of desmedipham. However the CLH report is an independent hazard assessment of desmedipham and therefore in some cases the conclusions in the CLH report are different from those in RAR. While in general the CLH report is based on the study summaries in RAR, in some cases data from the original study reports have been looked and included, especially in cases where there is no summary of this data in RAR.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Purified substance: crystalline powder Purified substance: colourless Technical grade: Crystalline powder	1999 B.2.3/01 M-193646-01-1 M-193642-01-1	Colour may vary from off-white to light beige in natural light.
Melting/freezing point	118.5 °C	1999 B.2.1/01 M-186353-01-1	
Boiling point	No boiling point, decomposition begins at 234 °C. (98.1 % pure)	2012 B.2.1/02 M-440200-01-1	Test item boiled under decomposition in the temperature range of 295 to 305 °C.
Relative density	Relative density at 20 °C compared to water at 4 °C: $D^{20}_4 = 1.32$	2012 B.2.14/01 M-439464-01-1	
Vapour pressure	**Vapour pressure of active substance:** 1×10^{-8} Pa at 20 °C 4×10^{-8} Pa at 25 °C 1×10^{-7} Pa at 30 °C	1990 B.2.2/01 M-146570-01-1	Estimated values from measurements at 86 °C, 90 °C and 98 °C
Surface tension	*72.39 mN/m at 20 °C*	1999	The surface tension of

Property	Value	Reference	Comment (e.g. measured or estimated)
	(6.3 mg/L in distilled water)	2012 B.2.12/01 M-186275-01-1 M-442960-01-1	desmedipham (c = 5 mg/L) stabilized in 7.0 mmol/L acetic acid: 71.5 mN/m at 20 ± 0.5 °C Due to the lack of stability of desmedipham in solutions with pH > 4, the test was performed in distilled water with small amounts of acetic acid. The surface tension of the blank solution was σ = 71.8 mN/m.
Water solubility	7 mg/L at 25 °C (pH=4) (99.6 % pure) **Water solubility of active substance:** 5.6 mg/L at 20 °C (pH = 6.2)	2012 B.2.5/01 M-442966-01-1	
Partition coefficient n-octanol/water	$LogP_{o/w}$ = 3.39 at 22 °C (pH=3.9) (shake flask method) **n-octanol/water partition coefficient of active substance:** $LogP_{o/w}$ = 2.7 at 25 °C (pH = 4, 7, 9) $P_{o/w}$ = 500 at 25 °C (pH = 4, 7, 9)	1987 2012 B.2.7/01 M-146516-01-1 M-427376-01-1	The results show that the partition coefficient of desmedipham, pure substance, is not pH dependent.
Flash point			Not required as the melting point of the active substance is higher than 40 °C.
Flammability	Not flammable	1995 B.2.9/01 M-146600-01-1	
Explosive properties	No explosive properties	2012 B.2.11/01 M-440204-01-1	
Self-ignition temperature	No self-ignition was registered up to a temperature of 120 °C. The temperature was not raised to 400 °C due to the low melting point of the substance.	1995 B.2.9/02 M-146600-01-1	
Oxidising properties	No oxidizing properties	1995	

Property	Value	Reference	Comment (e.g. measured or estimated)
		B.2.13/01 M-146600-01-1	
Granulometry	-		
Stability in organic solvents and identity of relevant degradation products	-		
Dissociation constant	No dissociation found in the pH range of 2 < pH < 6. At pH values above pH 7 a rapid hydrolysis of the substance takes place.	2012 B.2.8/01 M-442862-01-1	
Viscosity	-		

8 EVALUATION OF PHYSICAL HAZARDS

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

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

Absorption:

Based on urine excretion data on the ethyl phenyl carbamate (EPC) ring radiolabelled compound, desmedipham was well absorbed from the gastrointestinal tract after low doses (1990, RAR B.6.1.1/02). Overall, the absorption was about 80% (79-86 % in 96 hours). The excretion of phenyl carbamate (PC) ring radiolabelled desmedipham was somewhat slower, up to 62 % in 24 hours after a single low dose and 73-83 % after repeated low doses within 24 hours. The absorption was less complete and more slow after a single high dose. Only about 33% of PC ring radiolabelled desmedipham was absorbed in the first 96 hours as evidenced by urinary excretion after a single dose of 1000 mg/kg (1993, RAR B.6.1.1/04).

Excretion:

Urine was the main route of excretion after single and repeated low doses (1995, RAR 6.1.1/01; 1990, RAR B.6.1.1/02; 1993a, RAR 6.1.1/03). Overall, for the EPC ring, the urine excretion was about 80% (79-86 % in 96 hours). After a single high dose the majority, 51% of EPC radiolabelled dose, was eliminated in faeces and 43% in urine within 96 hours. The total excretion was nearly 100% within 96 hours of EPC ring labelled desmedipham.

PC ring radiolabelled desmedipham was excreted slower and to a lesser extent than EPC ring labelled active substance. After a single high dose the excretion was slower than after low doses. The majority, 56% of PC radiolabelled dose was eliminated in faeces and 33% in urine within 96 hours. After single or repeated low doses, urinary excretion was between 66 - 74% of PC ring radiolabelled dose within 96 hours. The total excretion was 71% in males and 65% in females within 24 hours and about 93% in both sexes within 96 hours.

The elimination half–life of the PC label in rat blood was 70 – 116 hours. The half-life for EPC ring label activity was not directly available, but excretion data indicates that it was significantly shorter than the half-life for the PC label.

Distribution:

Desmedipham is distributed mainly to organs with high blood flow (1993b; RAR 6.1.1/05). The main target organs/tissues were (in the order of magnitude) blood, plasma, liver, lungs, kidneys, heart, spleen, muscle, ovaries, testes, renal fat, thyroids and adrenals. Residues of desmedipham were also detectable in bone, brain and eyes.

After single or repeated low doses of EPC ring radiolabelled desmedipham, the residue levels in blood and plasma were very low (0.01 mg eq/kg) and below the limit of detection in other tissues 30 and 96 hours after dosing. Residue levels became higher after the single high dose of EPC ring radiolabelled desmedipham in blood (3.0 - 4.0 mg eq/kg), liver, lungs, kidneys, heart and plasma (0.5 - 0.7 mg eq/kg) 96 hours after dosage.

After single or repeated low doses of PC ring labelled test substance, the residue levels e.g. in liver, lungs, kidneys, heart, and especially in blood and plasma were over 10 to 100 times higher (0.4 - 1.0 mg eq/kg) than after similar EPC radiolabelled treatment. After a single high dose of PC ring radiolabel, the blood and plasma values were at least 30 times higher (123 - 170 mg eq/kg and 100 - 119 mg eq/kg) and in the other main target organs about 10 times higher than after similar EPC radiolabelled dose. The difference is explained by the much slower elimination rate of the PC ring derived metabolites.

Peak concentrations (Cmax) in blood were found 2 and 12 hours after dosage for the single low and high dose levels, respectively, in males. Females exhibited higher residue levels in blood 24 hours after a high dose. In plasma, the terminal half-life of the PC ring labelled radioactivity was shorter (39 - 59 hours) than in the blood (70 - 116 hours) and the percentages of the administered dose smaller than the corresponding blood values. Thus the clearance of PC radiolabelled desmedipham was slower from red blood cells than from plasma. The levels of radioactivity in tissues decreased gradually, but quantifiable residues were detected 7 days after the low dose and even 9 days after the high dose of PC radiolabelled desmedipham.

There was evidence of slight differences between sexes in absorption, distribution and elimination of test material. Radioactivity levels in blood and plasma reached maximum values later in females than in males, especially after the high dose level. In general, radioactivity levels in female tissues were higher than those observed in males.

Metabolism:

Desmedipham was rapidly metabolised via oxidative/hydrolytic cleavage of the parent compound (1990, RAR B.6.1.1/02; 1993, RAR 6.1.1/06). The major metabolites were N-(3-hydroxyphenyl)ethyl carbamate from the EPC ring radiolabelled form and 4-acetamidophenol from the PC ring radiolabelled form. N-(3-hydroxyphenyl) ethyl carbamate was partially converted into 3-aminophenol with subsequent acetylation to 3-acetamidophenol.

In the proposed metabolic pathway, the first metabolite of the PC ring radiolabelled form was PMC (phenyl methyl carbamate), which was supposed to convert to aniline and then further rapidly to 4-aminophenol and at last acetylated to 4-acetaminophenol. However, aniline was not detected in metabolism studies in rat.

The major metabolites of both EPC and PC ring radiolabelled test material were detected as conjugates in urine and in free form in faeces. Unchanged parent desmedipham was not excreted in urine, but found in faeces especially after a high dose of PC ring radiolabelled desmedipham. For both PC and EPC ring labelled test material, there did not appear to be any sex differences in urinary metabolites.

Comparative inter-species *in vitro* metabolism was performed in in vitro systems by incubating the test item with liver microsomes from male Wistar rats and humans with test durations of 0.5 and 1 hour (2015, RAR B.6.1.1/08). The identity of metabolites was not determined. The results of the study demonstrated a slightly different metabolic pattern of ^{14}C-Desmedipham when comparing rat and human liver microsomes. This

observation belongs however only to the lower number of metabolites detected in human liver microsomes. All metabolites in human liver microsomes, were also detected in the tests with rat liver microsomes.

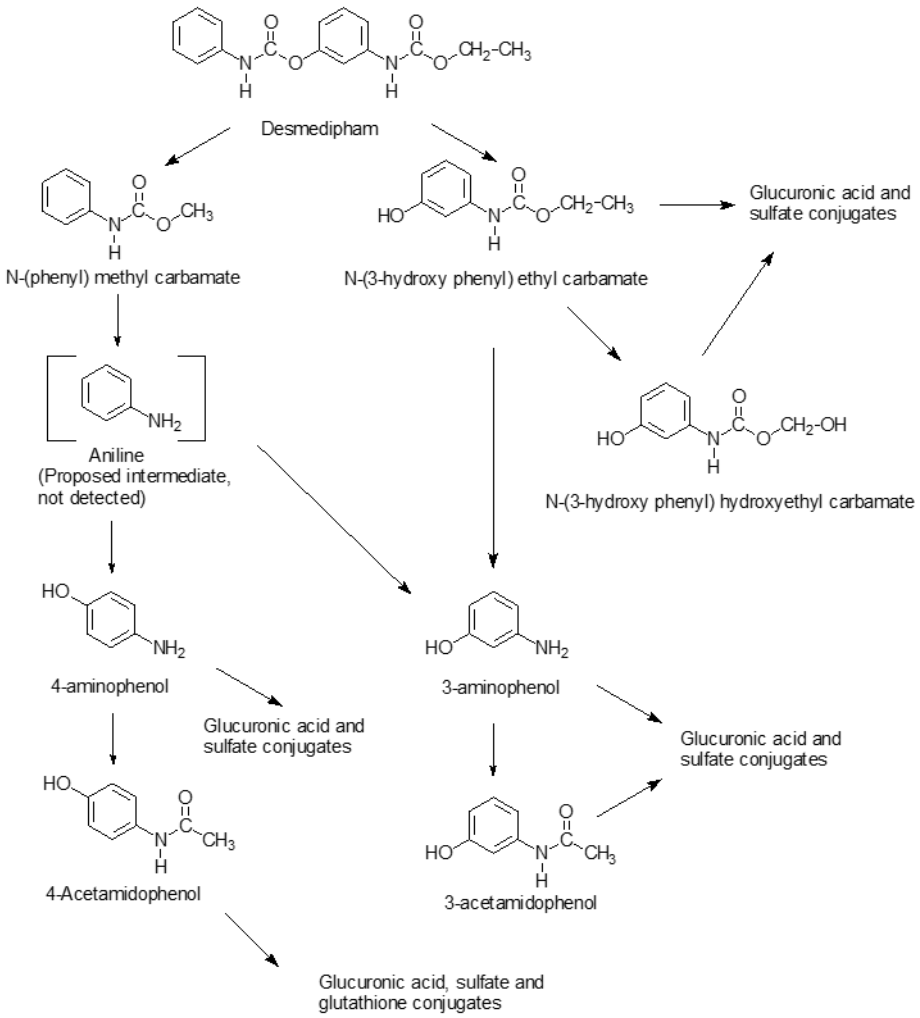


Figure 1 Metabolic pathway

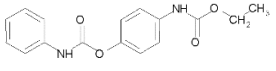
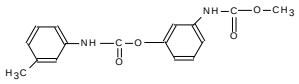
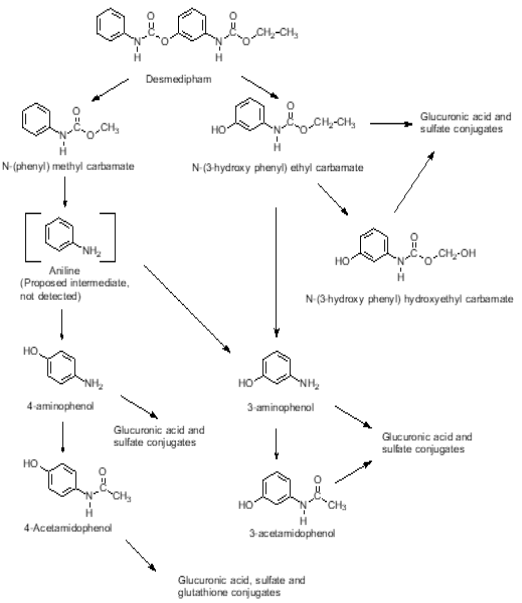
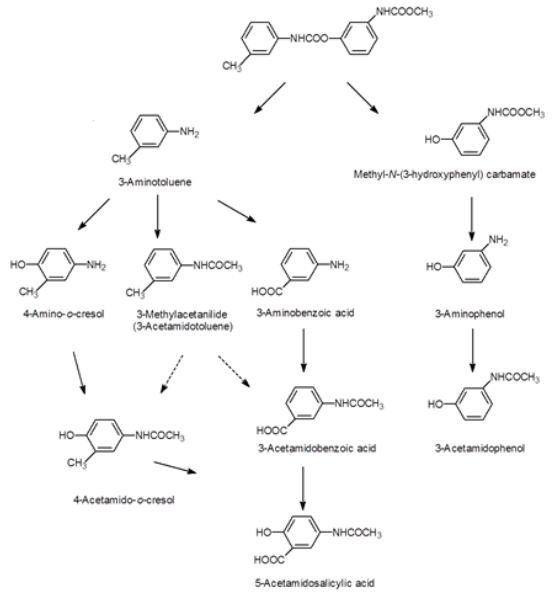
10 EVALUATION OF HEALTH HAZARDS

Read-across justification

The classification proposal is based on the data on desmedipham itself supported by read-across from phenmedipham and their assumed common metabolites.

The chemical structure, chemical properties, breakdown products and toxicological profiles of desmedipham and phenmedipham are similar. Desmedipham differs from phenmedipham by one additional methyl group in the carbamate and one less in the phenyl ring.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DESMEDIPHAM (ISO); ETHYL 3-PHENYLCARBAMOYLOXYPHENYLCARBAMATE

Chemical name	**Desmedipham**	**Phenmedipham**
IUPAC	ethyl 3-phenylcarbamoyloxyphenylcarbamate	methyl 3-(3-methylcarbaniloyloxy)carbanilate; 3-methoxycarbonylaminophenyl 3′-methylcarbanilate
CAS	13684-56-5	13684-63-4
Structural formula		
Molecular weight	300.3 g/mol	300.3 g/mol
Metabolic pathway		

Based on the data from available toxicokinetic studies desmedipham and phenmedipham are well absorbed from the gastrointestinal tract after low doses. Substances were widely distributed in the body mainly to organs with high blood flow. The main target organs/tissues were blood, plasma, liver, lungs, kidneys, heart, spleen, muscle, ovaries, testes, thyroid gland and adrenals. No indications of accumulation were noted. The administered doses were excreted fairly rapidly (urinary and fecal excretion).

There are slight differences in the substances formed during metabolism between the two substances. The first step of metabolism pathways seem to be slightly different. The –NHCOO- group in between the aromatic rings is metabolised in the first step to –NH2 and HO- in phenmedipham and to –NHCOOCH3 and HO- for desmedipham. However, both substances are suggested to produce compounds which have aromatic amine structure. Some of the identified metabolites are common for both substances such as 3-aminophenol and various acetamidophenols. Phenmedipham is also suggested to metabolise to acetamidocarboxylic and salicylic acids, which are not identified in the toxicokinetic studies of desmedipham. Not detected in the studies but phenmedipham is also suggested to produce aniline.

Desmedipham and phenmedipham are metabolised to compounds which have aromatic amine structure. It is well known that aromatic amines have potential to induce formation of methemoglobin. Methemoglobin is a transformation product of normal oxyhemoglobin caused by the oxidation of Fe^{2+} to Fe^{3+}, thus converting ferroprotoporphyrin to the ferriprotoporphyrin form. MetHb binds oxygen more strongly than Hb and

therefore does not effectively deliver oxygen to tissues. Based on available information on metabolites, 3-aminotoluene and aniline have potential to induce methemoglobin conversion and other hematotoxic effects (ECHA dissemination website). 3-aminotoluene (CAS 108-44-1, m-toluidine) has a harmonized classification as STOT RE 2 (effects on blood). Aniline (CAS 62-53-3) has a harmonized classification as STOT RE 1 (effects on blood), Carc cat 2 and Muta. cat 2. In addition, 3-aminophenol (CAS 591-27-5, p-aminophenol) and 4-aminophenol (CAS 123-30-8, m-aminophenol) have shown slight effects on blood (ECHA dissemination website; SCCS, 2011; SCCP, 2006).

The similarity of the effects seen in the toxicity studies seem to indicate that differences in the substances formed during the metabolism are not significant regarding their toxicity profiles. Desmedipham and phenmedipham in rats, mice and dogs show same type of effects on blood. The effects observed for both substances are consistent with effects pointing towards methemoglobinemia, leading to changes in red blood cell parameters and slight hemolytic anemia, increased activities of the bone marrow, kidney, liver and spleen - the organs mainly involved in the turnover of red blood cells - and compensatory hematopoiesis. For more detailed information see section 10.12 (specific target organ toxicity – repeated exposure). The similarity in toxic effects is also supported by the similar level LOAEL-values of the higher tier studies (repeated dose toxicity and chronic toxicity). The available data do not indicate significant quantitative differences in potency between substances. They seem to share a similar toxicity on blood and it is plausible they also share the same toxic mode of action.

The read-across is proposed to be used as a supporting evidence for classification desmedipham and phenmedipham as STOT-RE 2 based on effects in blood. Moreover, the read-across is proposed to be used as a supporting evidence for reproductive toxicity and carcinogenicity classification

Toxicology comparison of desmedipham and phenmedipham

Endpoint	Desmedipham	Phenmedipham
Acute oral	>2000 mg/kg (rat)	>2000 mg/kg (rat)
Acute inhalation	>7.4 mg/l (rat)	> 7 mg/l (rat)
Acute dermal	>2000 mg/kg (rat)	> 2500 mg/kg (rat)
Irritation – skin	No	No
Irritation - eye	No	No
Sensitisation	Slightly sensitising (GPMT)	No (study not acceptable)
Repeated dose toxicity (oral, rat and dog)	Rat (90 day oral, Wistar) LOAEL 5.2-5.6 mg/kg bw/day (methemoglobinemia) Rat (90 day oral, Wistar) LOAEL 24-27 mg/kg/bw day (↑ methemoglobinemia, ↑ hematopoiesis in the liver and spleen) Mice (28 day oral, NMRI) LOAEL 22-26 mg/kg/bw/dat (methemoglobinemia/haemolytic anemia, Heinz bodies ↑, hematopoiesis in spleen) Dog (90 day oral, Beagle) LOAEL 21.1. - 56.7 mg/kg bw/day (↑ Minimal/mild follicular epithelial hypertrophy in thyroids and increased thyroid weight) Dog (90 day oral, Beagle) LOAEL 53-57 mg/kg bw/day (marked iron deposits in the liver, ↑ erythropoiesis in the bone marrow)	Rat (90 day oral, Fischer 344) LOAEL 35 – 37 mg/kg bw/day (hemosiderin deposition (spleen), enlarged and/or black spleen, organ weight changes (spleen ↑, adrenals ↑), minimal anemia (reduced Hb, Hct, RBC; increased number of reticulocytes), changes in WBC (lymphocytes, neu-trophils). Rat (90 day oral, Sprague-Dawley) LOAEL 30-33 mg/kg bw/day (mild decreases of red blood cell parameters (Hb, Hct, RBC), hemosiderin deposition (spleen, liver, kidneys), extramedullary hematopoiesis in the spleen, organ weight changes (spleen ↑ in males, uterus and thymus ↓ in females) Mice (8-week oral, Swiss CD-1) LOAEL: 623-699 mg/kg bw/day (methaemoglobinemia,increased liver weights (males), brown pigment in hepatic Kupffer cells, reduced Hb, Hct, RBC)

		Dog (60 day oral, Beagle) LOAEL 11-12 mg/kg bw/day (reduced BW gain in males, organ weight changes (thyroid ↑, testes ↓ ovaries ↑), possibly thyroid hypertrophy.
Genetic toxicity	Negative Ames, In vitro: OECD 476 +, OECD 473 (+/-), In vivo: OECD 474: negative, no germ cell tests	Negative Ames, Negative OECD 476, Positive OECD 473 (2), OECD 474 (+/-), negative OECD 483 (exposure not shown)
Carcinogenicity	Rat LOAEL 300 ppm (15.7 mg/kg bw/day) ↑ incidence of pituitary adenomas in Han Wistar males Mice LOAEL 30 ppm (5.8 mg/kg bw/day) ↑ incidence of ovarian tubular adenomas	Rat LOAEL 500 ppm (34 mg/kg bw/day) ↑ incidence of endometrial stromal sarcoma (Sprague-Dawley) ↑ incidence of pituitary adenomas in Han Wistar males (500 ppm/2500 ppm) Mice: no increase in tumour incidences
Reproductive toxicity	Sprague Dawley, 2-gen: Maternal BW ↓ at 250 and 1250 ppm sperm (P) ↓ at 1250 ppm Developmental: ↑ motor activity, delayed eye opening and decreased sperm count at 250 and 1250 ppm, delayed onset of puberty at 1250 ppm.	Sprague Dawley, 2-gen: Maternal BW gain ↓ and FC ↓ at 1000 ppm (86 mg/kg bw/day) Pups: none (minor decreases in BW/BW gain were observed) Reproduction: none
	Wistar, 2-gen: Parents: BW and food consumption ↓ at 1250 ppm. Hemolytic anemia, splenic weights ↑, erythropoiesis or hemosiderosis in spleen, erythroid hyperplasia in bone marrow, follicular hyperplasia in thyroid at 250, 1250 Reproduction: reduced litter size at 250 and 1250 ppm Pups: F1A BW ↓ of at 250, 1250 ppm	Wistar:, 2-gen: Maternal BW/BW gain ↓ at 25 mg/kg bw/day Pups: Reduced BW (both sexes) at 1000 ppm (75 mg/kg bw/day) during lactation Reproduction: none
Developmental toxicity	Wistar: Maternal NOAEL >500 mg/kg Developmental: Increase in incidence of infarct of liver, bipartite ossification of sternebra at 100 and 500 mg/kg bw/day Increase in incidence incomplete ossification of interparietal bone and at 500 mg/kg bw/day (NOAEL 10 mg/kg)	Wistar: Maternal NOAEL <516 mg/kg): Reduced (corrected) BW gain and FC Pups (NOAEL <516 mg/kg): Reduced BW and incomplete ossification
	Wistar: Maternal NOAEL 10 mg/kg bw/day based on reduced body weight and corrected body weight gain, and reduced food consumption at 1000 mg/kg bw/day and slight ↓ bw gain at 100 mg/kg bw/day Developmental: ↑ number of fetuses with supernumerary rib at 100 mg/kg bw/day, (agnathia, microagnathia) observed at 100 and 1000 mg/kg bw/day	Wistar: Maternal NOAEL <150 mg/kg:based on reduced corrected BW gain Pups NOAEL <150 mg/kg): Runts were observed in all treated groups but not in controls
	Sprague Dawley: Maternal NOAEL 60 mg/kg bw/day: increase of spleen weight and	

	discolored urine at 250 and 1000 mg/kg bw/day. Developmental NOAEL 60 mg/kg bw/day: Increase in incidence of Subcutaneous haemorrhage at 250 and 1000 mg/kg bw/day. Fetal weight reductions, delayed ossifications at 1000 mg/kg bw/day	
	NZW rabbit: Maternal NOAEL 30 mg/kg bw/day: ↓ body weight and food consumption at 270, ↑ spleen weight at 90 and 270 mg/kg bw/day Developmental NOAEL 30 mg/kg bw/day: ↑ percentages of early embryonic death, fetal body weight ↓ at 270	NZW rabbit: Maternal NOAEL 225 mg/kg bw/day: BW gain ↓ and FC ↓ at 1000 mg/kg bw/day Developmental NOAEL 225 mg/kg bw/day: Reduced BW and retarded ossification at 1000 mg/bw/day

Acute toxicity

10.1 Acute toxicity - oral route

10.2 Acute toxicity - dermal route

10.3 Acute toxicity - inhalation route

10.4 Skin corrosion/irritation

10.5 Serious eye damage/eye irritation

10.6 Respiratory sensitisation

10.7 Skin sensitisation

10.8 Germ cell mutagenicity

10.9 Carcinogenicity

Table 9: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**		**Reference**
		NOAEL	**LOAEL** **Effects**	
24-month oral toxicity study in rats OECD 452 (1981), GLP. Rat, Sprague-Dawley 70/sex/group Microscopial	desmedipham technical, purity: 98% 0, 100, 400, 1200 ppm M: 0, 5.4, 21.6,	100 ppm M: 5.4 mg/kg bw/day F: 6.9 mg/kg bw/day	400 ppm Hematological effects; ↓ RBC parameters and related histopathological effects in spleen, liver and kidneys (pigmentation), urothelial	1991a, 1991b RAR B. 6.5/01 and B. 6.5/02 (chronic toxicity phase and carcinogenicity phase,

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results		Reference
		NOAEL	LOAEL Effects	
examinations of seminal vesicles, vagina, submandibular lymph node, tongue and nasal cavity were not included.	64.4 F: 0, 6.9, 28.4, 86.5 mg/kg bw/day Continuous in diet over 104 weeks		hyperplasia in kidneys	respectively) M-146979-01-1 M-146980-01-1
24-month oral chronic toxicity/carcinogenicity study in rats equivalent to OECD 453 (1981), GLP Rat, Wistar KFM-Han 70/sex/group	desmedipham technical, purity: 97.8%-98.2% 0, 60, 300, 1500 ppm M: 0, 3.2, 15.7, 79.9 mg/kg bw/day F: 0, 3.9, 19.8, 100.5 mg/kg bw/day Continuous in diet over 106 weeks	60 ppm M: 3.2 mg/kg bw/day F: 3.9 mg/kg bw/day NOAEL carc: 300 ppm (15.7 mg/kg bw/day)	300 ppm ↑ mineral deposition and associated urothelial hyperplasia in the kidneys and hemosiderin deposition and extramedullary haemopoiesis in the spleen at mid or high dose levels. ↑ methemoglobin LOAEL carc: 1500 ppm slightly ↑ incidence of pituitary adenomas in males	1986a, 2000 RAR B. 6.5/03 M-146766-01-1 M-196354-01-1
80 week oral carcinogenicity study in mouse equivalent to OECD 451 (1981), GLP Mice, Crl:CD1(ICR) BR VAF 50/sex/group	desmedipham technical purity: 97.5% 0, 400, 1000, 2500 ppm M: 0, 61, 153, 402 mg/kg bw/day F: 0, 72, 178, 501 mg/kg bw/day Continuous in diet over 80 weeks	NOAEL M/F < 61/72 mg/kg bw/day	LOAEL = 1000 ppm (F) and <400 ppm (M) Hepatotoxicity (↑ hepatocyte necrosis, regenerative hyperplasia) associated with ↑ incidence of hepatocellular adenomas in both genders LOAEL carc: 1000 ppm slight dose related increase in pulmonary adenoma incidences in females.	1994 RAR B 6.5/04 M-147004-01-1 M-146981-01-1
24-month oral carcinogenicity study in mouse equivalent to OECD 451 (1981), GLP Mice, NMRI KFM-Han 60/sex/group deficiencies in reporting and inadequate	desmedipham technical, purity 97.8% - 98.2% 0, 30, 150, 750 ppm M: 0, 4.2, 22, 109 mg/kg bw/day F: 0, 5.8, 31, 145 mg/kg bw/day	150 ppm M: 22 mg/kg bw/day	LOAEL = 750 ppm (males) Hematological effects (changes in RBC parameters), hematopoiesis in the spleen ↓ hemoglobin and hematocrit values in females at 750 ppm. ↑ Heinz body formation. ↑ Methemoglobin formation. Hemoglobin levels and hematocrit. ↑ White blood cell	1986b RAR B. 6.5/05 M-146765-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**		**Reference**
		NOAEL	**LOAEL** **Effects**	
statistical analysis	Continuous in diet over 104 weeks		count was increased at 750 ppm in females. NOAEL carc: < 30 ppm (5.8 mg/kg bw/day) ↑ ovarian tubular adenoma in females	

NOAELs and LOAELs are from the RAR except for pituitary tumors (divergent conclusion)

Table 10: Summary table of other studies relevant for carcinogenicity

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
In vitro L5178 TK+/- Mouse lymphoma mutation assay In ompliance with OECD 476 (1984) GLP	Desmedipham technical (Purity 98.0%)	1st test: 0, 6.3, 12.5, 25, 50 and 100 µg/ml 2nd test: 20, 40, 60, 80 and 100 µg/ml with and without S9 No HCD	**Result: Positive in the presence and absence of S9** Dose-related increase of the mutation frequency observed in both repetitions with and without S9 and in the absence of remarkable cytotoxicity.	1985 RAR 6.4.1/04 M-146755-01-1
In vitro Micronucleus test in human lymphocytes OECD 487 (2016) GLP	Desmedipham technical (Purity 98.9%)	Main tests: -S9 (3 hours) 12.5, 125, 250, 275, 300, 325, 350 and 400 µg/mL +S9 (3 hours) 12.5, 125, 200, 225, 250, 275 and 300 µg/mL -S9 (20 hours) 12.5, 65, 75, 85, 95, 105, 115 and 125 µg/mL Additional main tests: -S9 (3 hours) 5, 50, 100, 125, 150, 175, 200, 225, 250, 275 and 300 µg/mL +S9 (3 hours) 5, 50, 100, 125, 150, 175, 200, 225, 250, 275 and 300 µg/mL	**Result: Positive in the presence and absence of S9** Desmedipham caused an increase in the induction of micronuclei in cultured human lymphocytes after 3 hours exposure both in the absence and presence of S9 but not after 20 hours exposure.	2016 RAR 6.4.1/11 M-587137-01-1
In vitro Chromosome aberrations in human lymphocytes OECD TG 473 (1983) GLP	Desmedipham technical (Purity 98.0%)	0, 10, 50 and 100 µg/ml +S9 0, 10, 50, 100 and 500 µg/ml -S9	**Result: Negative in the presence and absence of S9**	1985 DAR 2001 6.4.1.6 M-146750-01-1

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Quantitative and qualitative whole body autoradiography in male CD-1 mice after oral application of [^{14}C]Desmedipham non OECD guideline study non GLP	[^{14}C]Desmedipham	Single dose of 2000 mg [^{14}C]Desmedipham/kg bw 2 animals	Desmedipham exposure of bone marrow was shown in low amounts. About 20% of blood concentration were present in bone marrow and about 10% in compact bone in both animals at 8 hours post dosing indicating desmedipham bone marrow exposure in mice. The % of total dose found in bone marrow and compact bone was less than 0.01% of the administered radioactive dose.	2017 RAR 6.4.2/05
In vivo Micronucleus test in NMRI mice OECD 474 (1983) GLP	Desmedipham technical (Purity 98.3%)	Single dose of 5000 mg/kg bw 1000 not 4000 cells scored No HCD	**Result: Negative** No change in PCE/NCE. Exposure of the bone marrow with the test substance was not demonstrated.	1985. RAR 6.4.2/01 M-146757-01-1
In vivo Alkaline Comet Assay OECD 489 GLP	Desmedipham (Purity 98.9%)	Oral doses of 0, 500, 1000 and 2000 mg/kg bw 3 animals/sex/dose	**Result: Negative** Desmedipham did not cause a significant increase in DNA damage in liver and stomach relative to the concurrent vehicle control.	2017 RAR 6.4.2/04 M-592294-01-1
In vivo Rat micronucleus test OECD 474 (1997) GLP	Desmedipham (Purity 98.2%)	Single doses of 0, 500, 1000 and 2000 mg/kg bw 2000 not 4000 cells scored No HCD	**Result: Negative** No change in PCE/NCE. Exposure of the bone marrow with the test substance was not demonstrated.	2003 RAR Addendum 3, 6.4.2.3 M-243574-01-1

Table 50 includes only studies which are relevant to conclude on genotoxicity of desmedipham. All studies are available in RAR. ADME data suggests bone marrow has been exposed in the rat MN study (**6.4.2.3, 2003**). For mice, autoradiography study (**6.4.2/05, 2017**) suggests exposure of target tissue is likely in the MN study (**6.4.2/01, 1985**). Thus, even though there was a positive in vitro MN assay (**6.4.1/11, 2016**), two in vivo MN assays (**6.4.2/01, 1985** and **6.4.2.3, 2003**) were negative and therefore desmedipham is considered unlikely to be aneugenic or clastogenic in vivo (with the reservation that not all impurities were tested in adequate amounts in the in vivo studies). An in vivo mammalian alkaline Comet assay (**RAR B.6.4.2/04, 2017**) conducted as a follow-up to positive gene mutation assay in vitro (**6.4.1/04, 1985**) was negative. Desmedipham is therefore unlikely to be genotoxic in vivo.

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Four chronic toxicity/carcinogenicity studies are available on desmedipham (**Table 9**).

Chronic administration of desmedipham in rats and mice resulted mainly haematological effects, including increased levels of methaemoglobin and changes in red blood cell parameters, and organ weight changes and histopathological effects related to the observed haemolytic anaemia. The studies generally complied with an older OECD guideline (1981) and histopathological investigation of some organs were not conducted in the studies. These organs were most often lacrimal gland, Harderian gland, cervix, coagulating gland and vagina. In many of the carcinogenicity studies, information is lacking on the content of impurities in the applied batches of technical desmedipham. The studies are only described briefly in the text below. Further details are given in RAR.

The 2-year chronic toxicity and carcinogenicity study in Spraque-Dawley rats revealed no clear indication of neoplastic effects (**RAR B. 6.5/01 and 02, 1991a, b**). At the end of the study the incidences of hepatocellular adenomas were slightly higher in desmedipham treated males than in controls (incidences **2%, 4%, 4% and 4%** at 0, 100, 400 and 1200 ppm, respectively) but there were no differences in hepatocellular tumour incidences in females (RAR). In females, a few cases of follicular adenoma of thyroids were observed at 400 and 1200 ppm, compared to no recorded cases in the control group (incidences **0, 0, 7%, 2%** in 0, 100, 400 and 1200 ppm, respectively) but no corresponding preneoplastic changes were reported. There were no differences in the incidences of thyroid tumours in males. Thyroid was not weighed in this study. The incidence of interstitial cell adenoma in testes was slightly increased at 1200 ppm (incidences **2%, 2%, 0** and **8 %** at 0, 100, 400 and 1200 ppm, respectively). No indications of preneoplastic changes e.g. increase in interstial cell hyperplasia or in its severity were reported. Overall incidence of tumours did not seem to be affected in a dose dependent way, but no statistical analysis was performed. The study has limitations regarding the scope of the tissues examined microscopically.

The 2-year chronic toxicity and carcinogenicity study in Wistar Han rats (**RAR 6.5/03, 1986a**) suffered from insufficient histopathological examinations and statistical analysis of the microscopic necropsy data, which led to a later re-examination of selected histopathological findings in all animals. This re-examination resulted slightly different incidences of these histological findings. Only the findings of re-examination are reported and discussed here, whereas in RAR also original incidences of histopathological findings are reported. Statistically significant decreases in T4 levels were observed at high dose (1500 ppm) in males and in all desmedipham treated female groups compared to controls. In females, also decrease in T3 level was observed. C-cell hyperplasia of thyroid was observed at slightly higher incidence in desmedipham treated male groups (RAR). Yet, there were no indications of neoplastic effects in the thyroid. In male rats, two years dietary administration of desmedipham at doses 300 and 1500 ppm resulted slightly, but not statistically significantly increased incidences in adenoma of pars distalis of pituitary (incidences **32.8%, 28.6%, 47.1% and 40 %** at 0, 60, 300 and 1500 ppm, respectively, Table 11). However, the incidences were high in all groups including the controls and within the historical control range of this tumour type in the performing laboratory (**19.6% - 54.2%**, **Table 11**). In females there was a statistically significant positive trend with dosage in the incidence of animals bearing one or more mammary gland tumours (p= 0.0026, **Table 12**). This was due to a higher than concurrent control incidence of tumors at low and intermediate doses whereas at high dose the incidence of mammary tumors was comparable to controls. The incidence of follicular cysts was slightly higher in high dose (1500 ppm) females than in controls. Moreover, in uterus one squamous cell carcinoma at high dose and one schwannoma in each of the desmedipham treated groups were observed compared to zero incidences of these tumors in controls (**Table 12**).

Table 11: Selected incidences of microscopic leasons in male rats (all lesions are from the study RAR B. 6.5/03, 1986a)

	Males				**Remarks**
Feeding dose, ppm	**0**	**60**	**300**	**1500**	
Pituitary / No. animals examined	70	70	70	70	
Focal hyperplasia, pars distalis - minimal - slight	7 3 1	2 2 2	1 3 1	5 0 2	Includes only terminally killed and decedents during carcinogenicity phase of the study (n =50/group)

	Males								Remarks
- moderate - marked - severe Total	2 1 14		0 0 6		2 2 9		0 0 7		
One Adenoma, pars distalis	D	T	D	T	D	T	D	T	
Interim	0	1	0	2	0	1	1	2	
Terminal	9	13	8	10	13	19	17	8	
Total incidence (%)	23/70 (32.8)		20/70 (28.6)		33/70 (47.1)		28/70 (40)		HCD from the performing laboratory: range 19.6 - 54.2% (avg. 37.5%), 10 studies conducted 1981-1986, 49-100 animals examined /study.

D = Decedent, T = Terminal kill

Table 12: Selected incidences of microscopic leasons in female rats (all lesions are from the study RAR B. 6.5/03, 1986a)

	Females								Remarks
Feeding dose, ppm	**0**		**60**		**300**		**1500**		
Pituitary / No. animals examined	70		70		70		70		
Focal hyperplasia, pars distalis [b] - minimal - slight - moderate - marked - severe Total	1 5 0 1 0 7		2 3 4 1 0 10		1 1 3 1 1 7		2 2 4 1 0 9		Includes terminally killed and decedents during carcinogenicity phase of the study (n =50/group)
One Adenoma, pars distalis	D	T	D	T	D	T	D	T	
Interim	0	1	0	1	0	1	0	0	
Terminal	13	25	13	26	11	28	11	26	
Total incidence (%)	39/70 (55.7)		40/70 (57.1)		40/70 (57.1)		37/70 (52.8)		
One Adenocarcinoma, pars distalis	1		0		0		0		
Mammary gland / No. animals examined	70		70		70		70		
Diffuse lobular hyperplasia – minimal – slight – moderate – marked	27 16 4 0		18 12 8 1		22 16 3 2		32 16 3 1		Includes animals killed in interim and terminal sacrifices and decedents, n = 70/group

	Females				Remarks
– severe – total	0 47	0 39	1 44	0 52	
Focal lobular hyperplasia	2	0	0	2	
Focal atypical hyperplasia	4	4	8	5	
One Fibroadenoma[a]	6	10	14	2	
Two Fibroadenomas[a]	0	2	1	0	
Three Fibroadenomas[a]	0	0	1	0	
Total no. animals with fibroadenoma (%) [b]	6 (8.6)	12 (17.1)	16 (22.8)	2 (2.8)	HCD from the performing laboratory: range 6-44%, avg. 26%, 10 studies conducted 1981-1986, 49-100 animals examined/study
One Adenocarcinoma[a] (%)	0 (0)	1 (1.4)	5 (7.1)	3 (4.3)	HCD from the performing laboratory: range 0-10%, avg. 3.2% 10 studies conducted 1981-1986, 49-100 animals examined/study
Ovary / No. animals examined	70	70	70	70	
Follicular cyst - interim - terminal - total	 3 8 11	 2 9 11	 1 13 14	 1 20 21	Combined uni- and bilateral
Uterus / No. animal examined	70	70	70	70	
Focal squamous hyperplasia	1	1	0	3	
Squamous cell carcinoma	0	0	0	1	
Schwannoma	0	1	1	1	

[a] Including terminally killed and decedents during carcinogenicity phase of the study (no mammary gland tumors were reported in interim sacrifice)

[b] Significant positive trend with dosage, P = 0.0026

In a 80-week mice carcinogenicity study (**RAR 6.5/04, 1994**), liver toxicity demonstrated by increased liver weights and chronic hepatocyte necrosis in both sexes, and increased regenerative hyperplasia in males, was observed at 1000 ppm and 2500 ppm (corresponding to 153 and 402 mg/kg bw/day). In males, a slight increase in chronic hepatocyte necrosis was also seen at the lowest tested dose (400 ppm, 61 mg/kg bw/day). Hepatocyte necrosis showed a clear treatment- and dose-related increase in both incidence and severity being more pronounced in males. Mortality of animals at high dose (2500 ppm) was markedly higher (14 - 20%) than in the control group and especially high dose females died or were killed in extremis earlier than the control females. There was a statistically significant ($p<0.05$) trend in mortality at 2500 ppm when the sexes were combined. Body weight gain of high dose animals was significantly reduced at study termination in both males and females (16% decrease in males and 26% in females) indicating, together with increased mortality, that the maximum tolerated dose was exceeded. Body weight gain of 1000 ppm males was also

reduced (10.5% decrease) compared to controls, but this decrease did not reach statistical significance. Increased incidence of cardiovascular disease (auricular thrombosis, myocardial fibrosis**,** ventricular thrombosis) was reported to contribute to mortality of high doses animals. The rapourter of the study presumed these findings to be related to reduced liver function in high dose animals with consequent effects on the cardiovascular system.

At the end of the study the incidences of hepatocellular adenomas and total hepatocellular tumors were statistically significantly increased in high dose females (2500 ppm, corresponding to 501 mg/kg bw/day, **Table 13**). In males, the incidences of hepatocellular adenomas and total hepatocellular tumors were increased in desmedipham treated groups in a dose-dependent manner (**Table 13**). Benign pulmonary adenomas were increased in a dose related manner in desmedipham treated females (incidences **6%, 10%, 12%, and 18%** in control, 400 ppm, 1000 ppm and 2500 ppm, respectively). There were no differences in the incidences of pulmonary adenomas in males. Pulmonary adenocarcinomas showed no treatment relationship in either sex. The overall incidence of pulmonary tumours was increased in treated females, but the increase was not statistically significant. Total number of benign tumors seemed to be increased at 2500 ppm but no statistical analysis was performed (RAR). In this study only liver, lungs, kidneys and ovaries were microscopically examined from all test animals.

Table 13: Selected incidences of neoplastic microscopic leasons in 80-week carcinogenicity study in mice (RAR B. 6.5/04, 1994)

Sex	**Males**				**Females**			
Feeding dose, ppm	**0**	**400**	**1000**	**2500**	**0**	**400**	**1000**	**2500**
Found dead / killed in extremis	14/50	17/50	14/50	21/50	12/50	13/50	9/50	19/50*
Survival at week 80	72%	66%	72%	58%	76%	74%	82%	62%
Liver / Total no. examined	50	49	49	50	50	49	48	48
Chronic hepatocyte necrosis								
- minimal	1	5	19	0	1	0	31	6
- moderate	0	1	15	16	0	0	4	25
- marked	0	0	5	32	0	0	0	11
- total	1	6	39***	48***	1	0	35***	42***
Regenarative hyperplasia	0	0	1	4	0	0	0	0
Adenoma (%)	11 (22%)	14 (28%)	15 (30%)	19 (38%)	0	0	0	3** (6%)
Carcinoma	8	4	5	6	0	0	0	1
Total hepatocellular tumors (%)	19 (38%)	18 (36%)	20 (41%)	25 (50%)	0	0	0	4**
HCD	Published HCD of Charles River lab. : Crl:CD-1 BR (1995): adenoma 0-19.2%				Published HCD of Charles River lab. Crl:CD-1 BR (1995): adenoma 0-2%			
Lungs /No. animals examined	49	49	49	50	50	49	49	49
Alveolar macrophage accumulation (%)	2 (4%)	4 (8%)	6 (12%)	10 (20%)	4 (8%)	1 (2%)	4 (8%)	6 (12%)
Pulmonary adenoma (%)	13 (27%)	10 (20%)	11 (22%)	10 (20%)	3 (6%)	5 (10%)	6 (12%)	9 (18%)

Sex	**Males**				**Females**			
Pulmonary adenocarcinoma (%)	4	3	8	4	1	3	5	1
Total pulmonary tumors (%)	17 (34%)	13 (27%)	19 (39%)	14 (28%)	4 (8%)	8 (16%)	11 (22%)	10 (20%)
HCD					Published HCD of Charles River lab. Crl:CD-1 BR mouse **(**1995): 18-month studies 0-15.38%, avg. 6.5%. 12 studies conducted 1985-1991, 50-100 animals/study. 21-month studies 0-10%, avg. 6.25%. 7 studies conducted 1983-1990. 24-month studies 4-18.37%, avg. 9.8% (24-month study). 11 studies conducted 1981-1990, 49-100 animals/study			

* Statistical significance at P < 0.05 compared to control, **P <0.01, ***P<0.001

The 2-year mouse study (**RAR B. 6.5/05, 1986b**) has a lot of deficiencies in reporting and statistical analysis, e.g. the non-neoplastic findings were not analysed statistically and the neoplastic lesions observed in interim kill were not included in the statistical evaluation. Moreover, it remained unclear if all grossly visible lesions were examined microscopically and why the total number of examined animals/organs was lower than intended. The study revealed slight but not statistically significant increase in the incidences of ovarian tubular adenoma in desmedipham treated mice (incidences **2%, 13%, 15% and 8%** in control, 30 ppm, 150 ppm and 750 ppm, respectively). No historical control data from the performing laboratory is available (for any tumor type observed in this study). However, the incidences of tubular adenoma in all treated groups were over the range of published HC incidences (**0-4.4%, Table 14**). Moreover, the incidences of theca/granulosa cell tumours in ovaries were slightly increased in high (750 ppm) and intermediate (150 ppm) dose females and the incidence of ovarian cysts was increased in a dose dependent manner in treated females (**Table 14**). There were some indications that all observed nodules or cysts in ovaries were not microscopically examined.

Leydig cell tumours in testes were observed in one low dose (30 ppm) male and in three males at 150 ppm, but not in the control or high dose males (incidences **0, 2%, 6% and 0** in control, 30 ppm, 150 ppm and 750 ppm, respectively). The incidence of spermatocele in epididymis was higher in all treated males than in the control group (2 – 4 animals, compared to none in controls, RAR). There were some indications that all observed macroscopical findings in seminal vesicles were not identified in histopathology. In the liver, the incidence of hepatocellular tumours (adenomas/carcinomas) was slightly increased in all treated males (total incidences **7%, 17%, 13% and 10%,** in control, 30 ppm, 150 ppm and 750 ppm, respectively, **Table 14**). One metastatic hepatocellular carcinoma was reported in mid dose (150 ppm) male scheduled for terminal sacrifice. Hepatocellular hypertrophy was increased in high dose males and the incidence of Kupffer cell proliferation was slightly increased in both sexes at high dose (RAR). Some rare tumours were found at terminal necropsy. Mesothelioma in body cavities was observed in one high dose male, and malignant neurinoma was recorded in one male and two females at 150 ppm. The total number of animals with neoplasms was not affected.

Table 14: Selected incidences of microscopic leasons in 24-month carcinogenicity study in mice (RAR B. 6.5/05, 1986b)

	Females				**Remarks/ HCD**
Feeding dose, ppm	**0**	**30**	**150**	**750**	
Liver / no. examined	50	50	50	50	Includes animals scheduled for terminal sacrifice. No neoplastic findings were reported in liver of females sacrificed in interim (n=10/group)
Adenoma	2				
Mammary gland / no. examined	50	47	46	43	Includes animals scheduled for terminal sacrifice. No neoplastic findings were reported in mammary gland of females sacrificed in interim (n=10/group)
Adenocarcinoma (%)	2 (4%)	5 (11%)	5 (11%)	3 (7%)	
Adenocanthoma	3	1	1	0	
Ovary / no. examined	49	47	48	49	Includes animals scheduled for terminal sacrifice. No neoplastic findings were reported in ovaries of females sacrificed in interim (n=10/group)
Cysts, combined serous and haemorrhagic (%)	7 (14%)	7 (15%)	9 (19%)	13 (27%)	
Theca/Granulosa cell tumor (%)	4 (8%)	4 (9%)	11 (23%)	7 (14%)	Published HCD of NMRI mice: 0-17.8%, avg 3.8% (benign and malign included), 18 studies conducted 1981-1988, totally 862 females examined (Bomhard, 1993)*
Tubular adenoma (%)	1 (2%)	6 (13%)	7 (15%)	4 (8%)	Published HCD of NMRI mice: 0-4.4%, avg 0.5%, 18 studies conducted 1981-1988, totally 862 females examined (Bomhard, 1993)
	Males				**Remarks / HCD**
Feeding dose, ppm	**0**	**30**	**150**	**750**	
Liver /Total no. examined	59	59	60	60	Includes animals sacrificed in interim (n=10) and animals scheduled for terminal sacrifice
Adenoma	2	6	6	2	
Carcinoma	2	4	2	4	Includes two carcinomas reported in interim sacrifice at 30 ppm
Total hepatocellular tumors (%)	4 (7%)	10 (17%)	8 (13%)	6 (10%)	

*HC range stated in RAR by the applicant differs from the data presented by Bomhard 1993 (corrected here).

Pituitary

There was no clear dose response in the incidences of adenomas of pituitary pars distalis in Wistar Han male rats (incidences **32.8%, 28.6%, 47.1% and 40 %** at 0, 3.2, 15.7 and 80 mg/kg bw/day, respectively, **Table 11**). The in-life phase of the study (**RAR 6.5/03, 1986**a) was conducted over June 1983-July 1985. The incidence of adenomas of pituitary pars distalis ranged **19.6-54.2%** (mean 37.5%) in 10 studies conducted during 1981-1986 in the performing laboratory. According to the applicant the data is from the same laboratory and the same rat strain but no other details have been provided. The total incidences of this tumor type in all male groups were within this historical control range of the performing laboratory and the increase in incidences of mid and high dose groups compared to concurrent controls can be ragarded as marginal. There were no differences in the incidences of pituitary tumors or preneoplastic lesions in female rats in the

same study (**Table 12**, **RAR 6.5/03, 1986a**) or in other rat or mice studies with desmedipham (**RAR 6.5/01-02, RAR, 6.5/04-05**).

However, the study with the structurally and toxicologically related substance, phenmedipham, with Wistar Han rats revealed increase in the incidence of adenomas of pituitary pars distalis in males only (phenmedipham CLH report). In this study the incidence of adenomas in the pars distalis of the pituitary showed a dose-dependent increase that was found to be statistically significant (time-to-tumor method) at 118 mg/kg bw/day (total incidences 14% and 38%, at 0 and 118 mg/kg/d, respectively). The increase was most pronounced in prematurely decedent males and the adenomas were reported to cause mortality. Likewise to desmedipham, the remaining long-term studies in rats with phenmedipham were performed using Sprague-Dawley rats that have very high spontaneous incidence of pituitary adenomas (generally >50%) and no effect of treatment on pituitary neoplasms were observed. There are some indications that the pituitary tumors appeared earlier also in desmedipham treated males compared to controls. At 52 weeks interim sacrifice focal hyperplasia of pituitary pars distalis was observed in one male in each of the desmedipham treated groups compared to zero cases in the control group. Enlarged pituitary was observed in one high dose animal at 52 weeks and during the last year of the study this finding was more frequently observed in desmedipham treated males compared to controls (incidences 5%, 15%, 12% and 20%, at 0, 3.2, 15.7 and 80 mg/kg bw/day, respectively) being more common in the decedent animals. We conclude that the slightly increased incidences of adenomas of the pars distalis in mid and high dose males compared to concurrent controls may be related to desmedipham treatment.

Mammary gland

In female Wistar Han rats the incidences of mammary gland fibroadenomas were increased in low and mid dose groups compared to concurrent controls (incidences **8.6%, 17.1%, 22.8% and 2.8%** at 0, 3.9, 19.8 and 100.5 mg/kg bw/d, respectively, **Table 12**, **RAR 6.5/03, 1986a**). There were also few mammary gland adenocarcinomas in desmedipham treated groups (incidences **0, 1.4%, 7.1% and 4.3%** at 0, 3.9, 19.8 and 100.5 mg/kg bw/d, respectively) and a statistically significant positive trend with dosage in the incidence of animals bearing one or more mammary gland tumours (p= 0.0026, Table 12). There was no remarkable difference in the incidences of preneoplastic findings in the mammary gland (Table 12). The in-life phase of the study was performed over June 1983 - July 1985. The historical control range of the performing laboratory within 5 years of the conduction of the study is **6-44 %** for fibroadenomas and **0-10%** for adenocarcinomas. The observed incidences in the study are within this range. Mammary gland tumors are generally common in aged Wistar rats.

According to the rapporteur of the study the dose-response curve with low incidences at high dose did not appear to be the consequence of differential time of sacrifice by group or differences in sampling technique or food intake. We note that there were no significant differences in the incidences of mammary tumors or preneoplastic findings in other rat or mice studies with desmedipham nor in the studies with related substance, phenmedipham. Therefore, although there are some indications, e.g. pituitary, thyroid and ovary findings, for hormonal imbalance in studies with desmedipham we conclude that the slightly increased incidences of mammary gland tumors in low and mid dose female Wistar rats can not be considered treatment-related and are not relevant for classification.

Liver

The significantly increased incidences of hepatocellular adenomas and total hepatocellular tumours in both sexes in 80-week carcinogenicity in mice were clearly related to excessive liver toxicity demonstrated by chronic hepatocyte necrosis and increased regenerative hyperplasia (Table 13, **RAR 6.5/04, 1994**). In this study the maximum tolerated dose was exceeded at high dose in both sexes (402 and 501 mg/kg bw/day, males and females, respectively) and presumably also in mid dose males (153 mg/kg bw/day). Although there were hepatoxicity in other studies with desmedipham (**RAR 6.5/03, 1986a; 6.5/05, 1986b**), no significant differences in hepatic tumour incidences were reported. Due to these factors we consider the increased incidences of hepatocellular tumours in Crl:CD1 mice of low relevance for humans and not relevant for classification.

Lungs

At the end of 80-week mice carcinogenicity study the incidences of pulmonary adenomas were increased in desmedipham treated females in a dose-related manner (incidences **6%, 10%, 12%, and 18%** at 0, 72, 178 and 501 mg/kg bw/day, respectively, **Table 13**, **RAR 6.5/04**). According to RAR the incidences of benign tumors in this study were not tested by Peto/fatal incidence analyses. The pairwise tests did not reveal statistical significances between the incidences of adenomas in different groups. The in-life phase of the study was conducted over November 1991–June 1993. No historical control data from the performing laboratory is available but the applicant has submitted published historical data of pulmonary adenomas in the same mice strain (Crl:CD-1 BR) from the time frame corresponding to the study (Charles River laboratories 1995). The animals were supplied from the same laboratory (Charles River UK Limited). At high dose the incidence was slightly over the historical control range of this published data for Crl:CD-1 BR mouse (**0-15.38%**). There were no differences in the incidences of pulmonary adenomas in males and pulmonary adenocarcinomas showed no treatment relationship in either sex (**Table 13**). The total incidence (including adenomas and adenocarcinomas) of pulmonary tumours was increased in desmedipham treated females, but the increase was not statistically significant.

Histopathological findings in lungs include slightly increased incidences of accumulation of fluid in the thoracic cavity and an increase in alveolar macrophages in intermediate (153 mg/kg bw/day) and high dose (402 mg/kg bw/day) males. There were no differences in histological findings of lungs in females in this study (**Table 13**) but increased alveolar macrophages in response to desmedipham treatment were also reported in rat studies in both sexes (**RAR 6.5/01-03**). According to study reporter of the other rat study (**RAR 6.5/03, 1986a**) this effect may indicate a mild phospholipidosis, a widely reported condition in laboratory rodents, which has been associated with cationic amphiphilic compounds (reviewed by Anderson and Borlak 2006). There were no clear indications of oncogenicity in lungs in other studies with desmedipham or in studies with the structurally and toxicologically related substance phenmedipham. However, significantly increased incidence of pulmonary alveolitis in high dose (1000 ppm) female rats was reported in one carcinogenicity study with phenmedipham (phenmedipham **RAR B.6.5.1/05, 1988c)**.

The toxicokinetic studies in rats revealed generally higher levels of radiolabelled desmedipham in tissues of females than in males but no remarkable difference in metabolism of desmedipham between sexes was reported (**RAR B.6.1.**). Altogether, we conclude that it remains unclear whether the slight dose-dependent increases in pulmonary adenoma incidences in female mice are treatment related.

Ovary

A slight but not statistically significant increase in the incidences of ovarian tubular adenoma was observed in NMRI KFM-Han mice after two years desmedipham treatment (incidences **2%, 13%, 15% and 8%** at 0, 5.8, 31 and 145 mg/kg bw/day, respectively, **RAR B. 6.5/05, 1986b**, **Table 14**). The in-life phase of the study was conducted over July 1983–July 1985. No historical control data from the performing laboratory is available but the applicant has submitted published historical data of the same mice strain (NMRI (BOR: NMRI (SPF Han) from the time frame corresponding closely to the study (1981-1988, Bomhard, 1993). The animals were breeded in the different laboratory. The incidences of tubular adenoma in all treated groups were over the range of published HC incidences of this mice strain in other laboratory (**0-4.4%,** Bomhard 1993). In the same study the incidences of theca/granulosa cell tumours in ovaries were slightly increased in desmedipham treated groups compared to concurrent controls but were, except at mid dose, within the published HC range submitted by the notifier (incidences of theca/granulosa cell tumours **8%, 9%, 23% and 14%** at 0, .8, 31 and 145 mg/kg bw/day, respectively, Table 14). There was no clear dose-response in the incidences of either of these tumour types. No preneoplastic or hyperplastic changes in the ovaries were reported. No indications for oncogenicity in ovaries was observed in the 80-week carcinogenicity study in mice with desmedipham (**RAR B. 6.5/04, 1994**) or in rat studies with desmedipham.

Ovarian tubular adenomas generally develop in aging mice, when ovarian activity significantly declines and secretion of gonadotropins (FSH, LH) from the pituitary increases. Increased incidences of ovarian tumors may also be caused by hormonal modulators (anti-estrogens, selective estrogen receptor modulators) and ovarian toxicity (Alison and Morgan 1987, Dixon et al. 2014). There are some indications in the database suggesting that desmedipham may induce hormonal imbalance affecting ovaries. Ovarian weights (absolute and relative) were statistically significantly increased in mid dose females after 12 months in this study (31

mg/kg bw day, 215%-226%, **RAR B. 6.5/05, 1986b**). Moreover, the incidences of ovarian cysts were increased in a dose dependent manner in treated females (total incidences **14%, 15%, 19% and 27%** at 0, 5.8, 31 and 145 mg/kg bw/day). A slight increase in follicular cysts at high dose was also observed in rat study with desmedipham (**RAR B. 6.5/03, 1986a**). However, we note that in repeated dose toxicity studies or in generational reproductive studies similar effect on ovarian weights or any remarkable effects on ovarian histopathology were not reported (**RAR B. 6.3. and 6.6.**).

To conclude, although increases in incidences of ovarian tubular adenomas in desmedipham treated mice compared to concurrent controls were slight, there was no clear dose-response and no hyperplastic changes were reported in the ovaries, we conclude that this finding may be related to desmedipham treatment.

Other tumor types

A few cases of follicular adenoma of thyroids in desmedipham treated females were reported in rat carcinogenicity study (**RAR B. 6.5/01-02**, incidences **0, 0, 7%, 2%** in 0, 100, 400 and 1200 ppm, respectively). There were no corresponding preneoplastic changes in the thyroid in this study and no differences in the incidences of thyroid tumours in males. No neoplastic findings in the thyroid were reported in the other rat carcinogenicity study (**RAR B. 6.5/03**) or in the mice studies.

In the rat carcinogenicity study (**RAR B. 6.5/01-02)** the incidence of interstitial cell adenoma (Leydig cell adenoma) in testes was slightly increased at 1200 ppm (incidences **2%, 2%, 0** and **8 %** at 0, 100, 400 and 1200 ppm, respectively). No indications of preneoplastic changes e.g. increase in interstial cell hyperplasia or in its severity were reported. In mice, a slight increase in incidence of interstial cell adenoma was reported in one study at low and mid dose, but not at high dose (**RAR B. 6.5/05**, incidences **0, 2%, 6% and 0** in control, 30 ppm, 150 ppm and 750 ppm, respectively). No preneoplastic changes in testes were reported. We conclude that slight, not dose-related increases in incidences of these tumour types (thyroid follicular and interstial cell adenomas) can not be considered treatment-related and are not relevant for carcinogenicity classification.

Table 15: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Rat, Wistar KFM	Pituitary adenoma of pars distalis	No	No	No	single, in males only	No	oral (relevant for human)	possibly hormonal, relevant to humans
Mice, Crl:CD-1 BR	Lung adenoma	No	No	No	single, in females only	Yes	oral (relevant for human	not known
Mice, NMRI KFM-Han	Ovarian tubular adenoma	No	No	No	-	No	oral (relevant for human)	possibly hormonal, low relevance to humans (the tumour type does not occur in humans)

10.9.2 Comparison with the CLP criteria

According to CLP criteria for the purpose of classification for carcinogenicity substances are allocated to one of two categories (Category 1 and 2) based on strength of evidence and additional considerations (weight of evidence). Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance.

Substances in Category 1 (known or presumed human carcinogens) may be further distinguished as Category 1A (known to have carcinogenic potential for humans) or Category 1B (presumed to have carcinogenic potential for humans) carcinogens. Classification for Category 1A is largely based on human evidence and classification for Category 1B is largely based on animal evidence.

There are no human data available for desmedipham, thus classification for Category 1A is not possible for desmedipham.

Classification for **Category 1B** should be based on animal experiments (or human studies) for which there is sufficient evidence to demonstrate animal carcinogenicity i.e. **"a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites."**

Desmedipham treatment caused slight, not statistically significant increases in incidences of three benign tumour types in three tissues (pars distalis of pituitary, lungs and ovary) and in two species (rat and mice). According to CLP criteria slightly, not statistically significantly increased incidences of **benign** tumours do not warrant classification for Category 1B.

The placing of a substance in **Category 2** (suspected human carcinogens) is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies. Limited evidence of carcinogenicity in animal studies is defined as: **the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.**

In rat, two years desmedipham treatment caused marginal increases in the incidences of pituitary adenoma of pars distalis in mid and high dose males only. The incidences of this tumour type were not dose-related and were within HC range of the performing laboratory in all groups (incidences **32.8%, 28.6%, 47.1% and 40 %** at 0, 3.2, 15.7 and 80 mg/kg/d, respectively, HC range **19.6-54.2%)**. In mice, desmedipham treatment resulted slightly increased incidences in pulmonary adenoma and ovarian tubular adenoma in females. Incidences of pulmonary adenomas were increased in a dose-related manner compared to concurrent controls (incidences **6%, 10%, 12%, and 18%** at 0, 72, 178 and 501 mg/kg bw/day, respectively) and the incidence at high dose was slightly over the published HC range for this mice strain (Crl:CD-1 BR mice, **0-15.38%**). There was no clear dose-response in the incidences of ovarian tubular adenoma (incidences **2%, 13%, 15% and 8%** at 0, 5.8, 31 and 145 mg/kg bw/day, respectively). No historical control data is available from the

performing laboratory, but the incidences of this tumour type in desmedipham treated groups were over the published HC range for this mice strain in other laboratory (NMRI KFM-Han, **0-4.4%).** There is no in vivo evidence of genotoxic potential for desmedipham. The MoA of formation of these tumours has not been studied but the database suggests that desmedipham may disturb hypothalamus-pituitary-thyroid/gonad axis, which could promote formation of pituitary adenomas of pars distalis and ovarian tumours. This mechanism is relevant for humans.

These findings could warrant classification for category 2 on the basis of Category 2 criteria **c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; and (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs**. However, the following additional considerations are made (weight of evidence analysis):

- The incidences of pituitary adenomas of pars distalis in all Wistar KFM Han male groups were within the historical control range of the performing laboratory and the increases in incidences were not statistically significant compared to concurrent controls and can thus be regarded as marginal. There were no differences in pituitary adenoma incidences in female Wistar KFM Han rats or in mice studies with desmedipham.

- Ovarian tubular adenomas are common spontaneous tumour in aged mice. There were no clear dose-response in the incidences of ovarian tubular adenoma and the increases in incidences in desmedipham treated mice were not statistically significant. No preneoplastic findings in ovaries were reported. No indications for oncogenicity in ovaries were observed in other mice study or in rat studies with desmedipham. Ovarian tubular adenomas do not occur in humans (Alison and Morgan 1987). Hence, this finding has low relevance for humans.

- Crl:CD-1 BR mice has variable spontaneous incidence of lung adenoma. There were no differences in tumour incidences in males or in adenocarcinoma incidences in females, and in this study the maximum tolerated dose was exceeded in high dose females. There were no indications of oncogenicity in lungs in other studies with desmedipham.

These factors discussed above weaken the available evidence and decrease the level of concern regarding the carcinogenicity concern for humans. Therefore, we consider these findings borderline evidence between category 2 and no classification, but finally too weak and inconsistent and as such, not sufficient for category 2 classification. However, based on pituitary and lung adenomas classification of desmedipham for category 2 for carcinogenicity could be argued.

10.9.3 Conclusion on classification and labelling for carcinogenicity

No classification is proposed for carcinogenicity.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

There are four carcinogenicity studies for desmedipham, two in the rat and two in the mouse. According to the DS, desmedipham caused slight, not statistically significant increases in the incidence of (1) pituitary adenomas in male rats (B.6.5/03), (2) lung adenomas in female mice (B.6.5/04), and (3) ovarian tubular adenomas in mice (B.6.5/05). The DS considered this to be a borderline case between Category 2 and no classification, but taking into account several factors decreasing the concern, they proposed no classification.

Comments received during public consultation

Four MSCAs and 1 Industry association provided their comments.

The Industry association agreed with the DS's proposal and provided a position paper containing additional arguments in support of no classification.

One MSCA also supported no classification, asking the DS why they considered the pituitary tumours potentially relevant for classification despite the lack of statistical significance, lack of a dose-response relationship and lack of an increase above the historical control data (HCD) range. The DS clarified that pituitary tumours were seen in a study with a structurally related substance phenmedipham and that in the study with desmedipham they found some indications that the pituitary tumours appeared earlier in treated animals than in the controls.

The remaining 3 MSCAs were in favour of Category 2 mainly based on the pulmonary and ovarian adenomas.

Assessment and comparison with the classification criteria

The available carcinogenicity studies with desmedipham are summarised in the following table.

Carcinogenicity studies		
Type of study; Reference; Year	**Method**	**Observations**
Rat		
2-year chronic toxicity/ carcinogenicity, dietary B.6.5/01,02 1991	OECD TG 453 GLP Strain: Sprague-Dawley Doses: 0, 100, 400, 1 200 ppm; equivalent to 0, 5.4/6.9, 22/28, 64/87 mg/kg bw/d (m/f) 1-year: 20/sex/group 2-year: 50/sex/group	<u>Non-neoplastic findings</u> 1 200 ppm (64/87 mg/kg bw/d): • ↓ Hb (by up to 10 %); ↑ lymphocytes; ↑ total bilirubin (up to 1.9-fold week 26) • ↑ incidence of haemosiderin deposition in Kupffer cells and renal tubular cells (m), increased haemosiderin in the spleen, increased alveolar macrophages 400 ppm (22/28 mg/kg bw/d): • ↑ total bilirubin (males; 1.3-fold) • ↑ incidence of increased alveolar macrophages (male) 100 ppm (5.4/6.9 mg/kg bw/d): no adverse effects <u>Neoplastic findings</u> None (slight increase several of tumour types in either male or females discussed by the DS)
2-year chronic toxicity/ carcinogenicity, dietary B.6.5/03 1986	OECD TG 453 GLP Strain: Wistar Han Doses: 0, 60, 300, 1 500 ppm; equivalent to 0, 3.2/3.9, 16/20, 80/100 mg/kg bw/d	<u>Non-neoplastic findings</u> 1 500 ppm (80/100 mg/kg bw/d): • ↓ bw (females; by 13 %) • ↑ spleen weight (by ca. 20 %) • ↓ Hb (by ca. 10 % throughout the study), ↑ MetHb (to ca. 5 %), Heinz bodies, ↑ total bilirubin

	(m/f) 1-year: 10/sex/group 2-year: 60/sex/group	• ↓ T4, ↓ T3 (females) • ↑ incidence of haemosiderin deposition in the spleen, renal calculi, foci of alveolar macrophages; a number of other histopathological changes reported at the initial examination but not at re-examination (e.g. thyroid follicular cell hyperplasia) 300 ppm (16/20 mg/kg bw/d): • ↑ MetHb (to ca. 2 %) • ↓ T4 (females) 60 ppm (3.2/3.9 mg/kg bw/d): no adverse effects <u>Neoplastic findings</u> None (several tumour types discussed by the DS, including pituitary adenomas)
Mouse		
1.5-year carcinogenicity study, dietary B.6.5/04 1994	OECD TG 451 GLP Strain: CD-1 Doses: 0, 400, 1 000, 2 500 ppm; equivalent to 0, 61/72, 153/178, 402/501 mg/kg bw/d (m/f) 50/sex/group Only liver, lung, kidneys and ovaries were examined from all animals of the low and mid-dose group	<u>Non-neoplastic findings</u> 2 500 ppm (402/501 mg/kg bw/d): • Slightly reduced survival • ↓ bw (by ca. 10 %) • ↑ liver weight (relative by 87 %/43 %; m/f) • Chronic hepatocyte necrosis (almost all animals, moderate to marked); splenic haematopoiesis; auricular thrombosis (males), myocardial fibrosis (males) 1 000 ppm (153/178 mg/kg bw/d): • ↑ liver weight (males; relative by 23 %) • Chronic hepatocyte necrosis (majority of animals, minimal to moderate) 400 ppm (61/72 mg/kg bw/d): no adverse effects <u>Neoplastic findings</u> 2 500 ppm: • **Hepatocellular adenoma** (females) • Pulmonary adenoma (females) ≤ 1 000 ppm: no neoplastic findings
2-year carcinogenicity study, dietary B.6.5/05 1986	OECD TG 451 GLP Strain: NMRI Doses: 0, 30, 150, 750 ppm; equivalent to 0, 4.2/5.8, 22/31, 109/145 mg/kg bw/d (m/f) 1-year: 10/sex/group 2-year: 50/sex/group Limited reporting	<u>Non-neoplastic findings</u> 750 ppm (109/145 mg/kg bw/d): • ↓ bw (males; by ca. 10 %) • ↑ MetHb (to ca. 5 %), Heinz bodies (ca. 40 %) • ↑ spleen weight (females, absolute by 73 %) ≤ 150 ppm (22/31 mg/kg bw/d): no adverse effects <u>Neoplastic findings</u> None (several tumour types discussed by the DS, including ovarian tubular adenomas)

Rat carcinogenicity studies (B.6.5/02 and B.6.5/03)

The top doses in these studies were comparable, 1 200 ppm and 1 500 ppm, respectively. The

main toxic effect was haematotoxicity (Hb reduced by ca. 10 %, increased MetHb and Heinz bodies). The maximum tolerated dose in 90-day studies was around 4 000 ppm (body weight reduction by ca. 20 %, Hb reduction by ca. 15 %; B.6.3.2/02, /04). The top dose selection in these two carcinogenicity studies is considered acceptable.

The DS discussed several increases in tumour incidences, which are listed below (incidences are provided for the control, low, mid and high dose group, respectively). Only the pituitary adenomas were considered potentially relevant for classification by the DS. In study B.6.5/02 only premature decedents were examined histopathologically at the low and mid dose.

- Thyroid follicular cell adenoma (B.6.5/02): females 0/50-0/25-2/30-1/50; males no increase
- Leydig cell adenoma (B.6.5/02): 1/50-1/21-0/28-4/48; no increase in hyperplasia
- Pituitary adenoma (B.6.5/03): males 33 %-29 %-47 %-40 %; HCD 20-54 % (mean 38 %)
- Mammary gland fibroadenoma (B.6.5/03): 9 %-17 %-23 %-3 %; HCD 6-44 % (mean 26 %)
- Mammary gland adenocarcinoma (B.6.5/03): 0 %-1 %-7 %-4 %; HCD 0-10 % (mean 3 %)
- Uterine squamous cell carcinoma (B.6.5/03): 0 %-0 %-0 %-1 %

Taking into account the lack of statistical significance at the top doses, the lack of a clear dose-response relationship in most cases and the incidences remaining within a relevant HCD range, where available, it is questionable whether any of these increases is treatment-related. In addition, each of these increases is found in only one and not in the other of the two rat carcinogenicity studies using similar top doses. Therefore, RAC does not consider any of these findings relevant for classification.

Mouse carcinogenicity study (B.6.5/04)

Significant hepatotoxicity at the top dose of 2 500 ppm (moderate to marked hepatocyte necrosis in almost all animals, a 1.9-fold increase in liver weight in males) indicates that the MTD has been reached and possibly exceeded.

The increases in the incidence of hepatocellular adenomas at the top dose, although not statistically significant on pairwise comparison, are likely to be treatment-related in view of the marked hepatotoxicity. The histopathological findings in the liver are summarised in the following table.

Histopathological findings in the liver in study B.6.5/04

	Males				**Females**			
Dose (ppm)	**0**	**400**	**1 000**	**2 500**	**0**	**400**	**1 000**	**2 500**
No. of animals examined	50	49	49	50	50	49	48	48
Chronic hepatocyte necrosis								
– minimal	1	5	19	0	1	0	31	6
– moderate	0	1	15	16	0	0	4	25
– marked	0	0	5	32	0	0	0	11
– total	1	6	39	48	1	0	35	42

Regenerative hyperplasia	0	0	1	4	0	0	0	0
Hepatocellular adenoma	11	14	15	19	0	0	0	3
Hepatocellular carcinoma	8	4	5	6	0	0	0	1
Total hepatocellular tumours	19	18	20	25	0	0	0	4

There was also an increased incidence of pulmonary adenomas in top dose females: 3, 5, 6 and 9 out of 49-50 animals at 0, 400, 1 000 and 2 500 ppm respectively. The increase is not statistically significant on pairwise comparison and was not accompanied by non-neoplastic findings. There was no increase in pulmonary adenomas in males (incidences 13, 10, 11, 10 out of 49-50 animals).

Mouse carcinogenicity study B.6.5/05

The top dose of 750 ppm caused haematotoxicity (methaemoglobinaemia, Heinz body formation, increased spleen weight) and in males also body weight reduction by ca. 10 %. A dose of 1 600 ppm increased MetHb levels to 14 % and splenic weights by 60 % in male NMRI mice after a 28-day administration (B.6.3.1/01). In view of the haematotoxicity, the top dose in the carcinogenicity study is considered sufficiently high.

The DS discussed several increases in tumour incidences, which are listed below (incidences are provided for the control, low, mid and high dose group respectively). Only the ovarian tubular adenomas were considered potentially relevant for classification by the DS.

- Ovarian tubular adenoma: 2 %-13 %-15 %-8 %
- Ovarian theca/granulosa cell tumour: 8 %-9 %-23 %-14 %
- Leydig cell adenoma: 0 %-2 %-6 %-0 %
- Hepatocellular tumours (adenomas + carcinomas): males 7 %-17 %-13 %-10 %

Taking into account the lack of statistical significance at the top doses and the lack of a clear dose-response relationship, it is questionable whether any of these increases is treatment-related. In addition, no increase in ovarian or testicular tumours was reported at 2 500 ppm in the other mouse carcinogenicity study (B.6.5/04). Therefore, RAC does not consider any of these findings relevant for classification.

Genotoxicity

A brief overview of genotoxicity studies is provided in the Background Document. The mutagenicity hazard class was not open for public consultation and data was presented only as background information for carcinogenicity assessment in the CLH report. Desmedipham is unlikely to be genotoxic *in vivo*. Although there was one positive mouse lymphoma assay, one *in vitro* micronucleus test positive at cytotoxic concentrations and one equivocal *in vitro* chromosomal aberration assay, all *in vivo* assays (3 micronucleus tests and 1 comet assay in the liver and stomach) were negative.

Conclusion on classification

Out of the neoplastic findings in the available studies, RAC finds a sufficient indication of a treatment-related effect only for the hepatocellular tumours in the mouse study B.6.5/04. However, taking into account the lack of statistical significance on pairwise comparison, the excessive liver toxicity at the tumorigenic dose (marked chronic hepatocyte necrosis and liver

enlargement), lack of increase in hepatocellular carcinomas in males and lack of genotoxicity, RAC agrees with the DS that **no classification for carcinogenicity is justified**.

Supplemental information - In depth analyses by RAC

Overview of genotoxicity studies

The table below provides a brief overview of the genotoxicity studies with desmedipham available in the RAR of October 2017.

Type of study	**Reference (RAR); year**	**Result**	**Remarks**
In vitro			
Ames	6.4.1/01; 1990	Negative	
Ames	6.4.1/02; 1991	Negative	TA102 or *E.coli* WP2 not tested
Ames	6.4.1/03; 2014	Negative	
Mouse lymphoma assay	6.4.1/04; 1985	Positive ±S9 at doses causing some (but not excessive) cytotoxicity	
HPRT	6.4.1/05; 1991	Negative	
Chromosomal aberrations	6.4.1/06; 1985	Negative; the top concentration limited by solubility in ethanol	
Chromosomal aberrations	6.4.1/07; 1991	Equivocal +S9	
UDS	6.4.1/08; 1988	Negative	
UDS	6.4.1/09; 1990	Negative	
Rec-assay in *Bacillus subtilis*	6.4.1/10; 1990	Negative	
Micronucleus	6.4.1/11; 2016	Positive ±S9 at cytotoxic concentrations (CPBI reduction by 46-55 %)	
In vivo			
Micronucleus (bone marrow; mouse)	6.4.2/01; 1985	Negative; dose 5 000 mg/kg bw	
Micronucleus (bone marrow; mouse)	6.4.2/02; 1991	Negative; dose 2 000 mg/kg bw	Bone marrow exposure in CD-1 mice demonstrated in a separate study (B.6.4.2/05; 2017)
Micronucleus (bone marrow; rat)	6.4.2/03; 2003	Negative; dose 2 000 mg/kg bw	
Comet (liver, stomach; rat)	6.4.2/04; 2017	Negative; top dose 2 000 mg/kg bw	

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 16: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results				Reference
		Maternal and paternal NOAEL	Developmental NOEAL	Reproductive NOEAL	Effects	
Two generation reproductive toxicity study OECD 416 GLP Wistar rat 24/sex/group	Desmedipham, purity 99.8% 0, 50, 250, 1250 ppm (M: 0, 4, 21, 100 mg/kg bw/day F: 0, 3, 18, 102 mg/kg bw/day) Continuous in diet	Maternal: 50 ppm (3 mg/kg bw/day) Paternal: 50 ppm (4 mg/kg bw/day)	3 mg/kg bw/day	4 mg/kg bw/day	Parental: reduced body weight at 250 and 1250 ppm Reproductive: slightly reduced sperm count in cauda epididymis at 1250 ppm in P generation, and at 250 and 1250 ppm in F1 generation Developmental: increased motor activity in F2 female pups, slightly delayed eye opening at 250 ppm, delayed balanopreputial separation, delayed vaginal opening at 1250 ppm in F1 generation pups	2003 RAR B. 6.6.1/01 M-493944-02-1 and 1st, 2nd and 3rd amendment to the report Key study
Two generation reproductive toxicity study Non-OECD TG (US-EPA 83-4), corresponding to OECD 416 Limitations in reporting of data and statistical analysis. Dams cannibalized some pups or the whole litter; corpora lutea or implantation sites were not reported. GLP	Desmedipham, purity 97.8% 0, 50, 250, 1250 ppm (M: 0, 4, 20, 90 mg/kg/bw/day F: 0, 6, 30, 140 mg/kg bw/day) Continuous in diet	50 ppm (6 mg/kg bw/day for males and 4 mg/kg bw/day for females)	50 ppm (6 mg/kg bw/day for males and 4 mg/kg bw/day for females)	50 ppm (6 mg/kg bw/day for males and 4 mg/kg bw/day for females)	Parental: reduced body weight, reduced food consumption at 1250 ppm; hemolytic anemia, increased splenic weights/erythropoiesis or hemosiderosis in spleen, erythroid hyperplasia in bone marrow, follicular hyperplasia in thyroid at 250 and 1250 ppm Pups: reduced body weight and organ weight (kidney, heart, brain) Reproduction: reduced litter size in F2A at 1250 and in	1986 RAR B. 6.6.1/02 M-146764-01-1 Supplementary study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**				**Reference**
		Maternal and paternal NOAEL	**Developmental NOEAL**	**Reproductive NOEAL**	**Effects**	
Wistar rat (KFM-Han, outbred, SPF) 30/sex/group					F2B pups at 250 and 1250 ppm	
Two generation reproductive toxicity study OECD 416 but with deficiencies (histopathology, necropsy data, statistics) GLP (but deficiencies in reporting) Sprague-Dawley rat 28/sex/group Six dams lost their whole litter during the first days of lactation (F1: 1 litter at 400 ppm; F2: 2 litters at 0 ppm, 2 litters at 100 ppm, 1 litter at 1200 ppm). Dams of these litters were considered to have been deficient in terms of maternal care, with pups being cold, scattered and poorly fed. Survival data from the totally lost litters and two additional litters with low viability index (50 % and	Desmedipham, purity 89.7 % 0, 100, 400, 1200 ppm (Apr. doses, not stated in the study but extrapolated from Becker et al 1986: M: 0, 8, 30, 100 mg/kg bw/day F: 0, 10, 50, 140 mg/&kg bw/day) Continuos in the diet	Maternal: 50 mg/kg bw/day Paternal: 30 mg/kg bw/day	Maternal: 50 mg/kg bw/day Paternal: 30 mg/kg bw/day	30 mg/kg bw/day	<u>Parental</u>: reduced body weight gain at 1200 ppm, reduced seminal vesicle weights in F0 males at 1200 ppm <u>Pups</u>: decreased litter and pup weight at 1200 ppm	1991 RAR B.6.6.1/03 M-146991-01-1 Study not reliable and rejected from the CLH dossier

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results				Reference
		Maternal and paternal NOAEL	Developmental NOEAL	Reproductive NOEAL	Effects	
56%) at 1200 ppm from the F2 generation were excluded from the tables of the overall litter performance and from the final reproduction evaluation. There was low viality (50-65%) also in other litters: 2 liiters at 100 ppm in F1generation; 2 litters at 100 ppm and 1 litter at 400 ppm in F2 generation but these were included in the evaluation tables in the study report. Due to the high mortality for an unknown reason which was not treatment related and due to the poor reporting, it is concluded that this study is not reliable and therefore rejected from the CLH dossier.						

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

There are three 2-generation studies conducted in rat and assessed in RAR for desmedipham. Two of the older studies from the 1980's and 1990's were conducted according to either an old version of OECD TG

416 or according to a US guideline for registering pesticides (US-EPA). Both of the older studies have deficiencies in histopathology and reporting, and it was not possible to fully evaluate fertility, sexual development and effects potentially related to changes in hormonal system. Due to the unexplained losses of six whole litters during the first days of lactation and some other litters with low viability (50-65%) and due to deficiencies in reporting the study (**RAR B.6.6.1/03, 1991**) is considered unreliable and therefore rejected from the CLH dossier. The newest 2-generation study (RAR B.6.6.1/01, 2003) was performed according to the OECD 416 (2000), however also this study has some deficiencies. Despite of the aforementioned deficiencies two 2-generation studies are considered acceptable for classification purposes. The studies are described briefly here and further details are given in the RAR.

The newest 2-generation study (**RAR B.6.6.1/01, 2003**) is a key study performed using Wistar rats not assessed before in the PPP review programme. The study report was amended three times due to deficiencies in statistical reporting and due to correcting falsely reported F2 pup data from tests which were not performed due to terminal kill of F2 pups after weaning. A GLP study audit was requested for the study. The conclusion from the audit was that some deviations were observed by the monitoring authorities. However, the observed deviations were not considered to affect the validity of the results or the study.

The rationale for the dosing was not given in the study report. Furthermore it is not clear from the description of the study report how litter was modelled and if covariates were considered where appropriate as for example when analysing onset of puberty data. We also note that the historical control data provided in RAR includes the study under assessment.

There were no mortalities or clinical signs in the parental animals. Maternal toxicity was observed at 1250 ppm in P generation and at 250 and 1250 ppm in F1 generation based on decreased body weight during pre-mating, gestation and lactation. There were no effects on males in P or F1 generation during the premating period. There was a significant reduction in body weight in P generation males at mid (6-7%) and high (6-7%) dose during weeks 12-16 and 11-12, respectively. Decreased terminal body weights were observed in P generation males at 250 and 1250 ppm and at 1250 ppm in F1 females. There was no effect on food consumption in either generation animals at any dose. In P generation females there was a decrease (30-51%) in feed efficiency at 1250 ppm during weeks 1-10. Feed efficiency was significantly affected in F1 generation females during week 10 at 250 ppm (61% decrease) and during GD 0-7 at 1250 ppm (-28%).

In P1 generation males there was an increase in relative weight of testis (6-10%), epididymis (10-14%) and cauda epididymis (8-14%) at 250 and 1250 ppm desmedipham in the presence of decreased body weight. The main effects on other organ weights in adult animals were decreased kidney weight, increased spleen weight and thyroid weights observed primarily at 1250 ppm or otherwise the effect was small or without dose response.

The parameters fertility, gestation and pregnancy index were not affected up to the highest dietary concentration of 1250 ppm. Also the live birth index, the survival indices and the lactation index were comparable to the controls. Furthermore, the litter size was comparable between the control and the treated groups.

Reduced total sperm counts were observed in cauda epididymis at 1250 ppm in the P generation and at 250 and 1250 ppm in the F1 generation males (Table 17 and Table 18). The total cauda sperm counts were slightly but statistically significantly lower compared to the respective controls and in the F1 generation the reduction was dose-dependent. The homogenization resistant testis spermatid number was reduced slightly but statistically significantly in the P generation at all of the tested doses but the effect was not dose dependent. In the F1 generation there were no changes in the homogenisation resistant testis spermatid number at any dose. The percent of motile sperm was decreased at the highest dose in both generations and the change was statistically significant but small compared to the respective controls. There were no treatment-related changes in sperm morphology in either of the generation at any dose. There were no effects on male reproductive organ weights or histopathology.

The level of concern is high for the epididymal sperm count result. In rodents lower sperm count does not necessarily manifest in lower fertility because there is a large surplus of sperm but the same might not be true for humans. Whether the slightly reduced sperm counts should be considered adverse is however doubtful. There was a large variation in the sperm count numbers in total cauda epididymis between individual animals in control and treated animals indicated by a large standard deviation and reflecting biological

differences between animals. The historical control data provided in RAR includes the study under assessment. The sperm findings were not accompanied by adverse findings in the reproductive organs. It is thus concluded that based on the weight of evidence the sperm count observation is an isolated finding and the level of concern is reduced.

Table 17 Summary of sperm parameters in P generation

Parameters	**Doses (ppm)**			
P generation	**0**	**50**	**250**	**1250**
Total no. sperm observed SD	432.00 12.069	450.04 31.405	461.33 43.698	504.54 72.124
No. dead sperm SD	36.75 4.078	36.08 4.754	39.21 3.741	48.13 8.002
% dead sperm of total sperm % of control	8.50	8.01 (-5.8)	8.50 (0)	9.54 (+12.2)
No. motile sperm SD	395.25 13.995	413.96 29.223	422.13 42.205	456.42 65.506
% motile sperm of total sperm SD % of control	91.48 1.057	91.97 0.908 (+ 0.54)	91.45 0.859 (-0.03)	90.46** 0.908 (-1.11)
Total cauda sperm number (x 10^6/g) SD % of control	1179.93 171.221	1145.45 112.880 (-3.03)	1163.57 130.042 (-1.39)	1058.29* 114.791 (-10.31)
Homogenization-resistant testis spermatid number (x 10^6/g) SD % of control	150.89 6.729	146.38** 5.695 (-2.99)	145.95** 4.052 (-3.28)	146.09** 5.233 (-3.19)

*p ≤ 0.05 **p ≤ 0.01

Table 18 Summary of sperm parameters in F1 generation

Parameters	**Doses (ppm)**			
F1 generation	**0**	**50**	**250**	**1250**
Total no. sperm observed SD	503.86 54.477	488.22 50.652	478.84 71.646	487.07 20.952
No. dead sperm SD	40.46 6.327	44.33 4.574	42.52 9.549	46.67 3.752
% dead sperm of total sperm % of control	8.03	9.10 (+13.32)	8.88 (+10.58)	9.58 (+19.30)
No. motile sperm SD	463.39 50.505	443.89 48.317	436.32 63.682	440.41 20.064
% motile sperm of total sperm SD % of control	91.97 0.856	90.89** 0.793 (-1.18)	91.32 2.001 (-0.71)	90.41** 0.741 (-1.70)
Total cauda sperm number (x 10^6/g) SD % of control	994.95 125.737	950.30 86.391 (-4.49)	914.32** 66.225 (-8.11)	895.13** 114.905 (-10.04)
Homogenization-resistant testis spermatid number (x 10^6/g) SD % of control	124.20 11.930	125.69 17.754 (+1.20)	124.53 14.093 (+0.26)	123.91 22.603 (-0.24)

*p ≤ 0.05 **p ≤ 0.01

Pup birth weight was slightly but significantly decreased in F1 pups at 250 ppm (3%). In F2 pups birth weight was decreased at 1250 ppm (8%). During lactation in the F1 generation pup body weight did not seem to be affected by treatment. In the F2 generation a statistically significant reduction (6-12%) of the pup weights during the lactation period was observed at 1250 ppm.

Of the physical development landmarks eye opening was slightly but significantly delayed in both males and females at 250 and 1250 ppm in the F1 generation (2.8-4.3%) (Table 19). In the F2 generation eye opening was significantly delayed at 250 and 1250 in females (1.4-2.1%), and at 1250 ppm only in males (2.7%). There was no consistent pattern of effects in other physical developmental landmarks (unfolding of pinna, tooth eruption, ear opening, hair growth) at any dose in either of the generation. The historical control data provided in RAR includes the study under assessment.

Table 19 Summary of eye opening in F1 and F2 pups

Eye opening (d)	Doses (ppm)			
	0	**50**	**250**	**1250**
F1 male pups SD	14.3 1.10	14.5 0.69	14.7** 0.70	14.7** 0.73
F1 female pups SD	14.0 1.20	14.0 1.45	14.4* 0.78	14.6* 0.76
F2 male pups SD	14.6 0.73	14.7 0.75	14.7 0.65	15.0** 0.72
F2 female pups SD	14.5 0.75	14.4 0.86	14.7* 0.63	14.8** 0.61

*p ≤ 0.05 **p ≤ 0.01

Vaginal opening was delayed at 1250 ppm in the F1 generation female pups but the delay was not statistically significant and the effect was not dose dependent (Table 20). Balano preputial separation was significantly delayed at 1250 ppm in the F1 generation male pups but the effect was not dose dependent. The body weights of the pups at the time of vaginal opening or balanopreputial separation in the F1 generation were not different from respective control. These parameters were not examined in the F2 generation pups due to terminal kill after weaning. In general, delays in preputial separation and in vaginal opening that are accompanied by delays in the onset of other developmental markers likely suggest an overall effect on growth and development. Furthermore, delay in these events in the absence of effects on body weight or other developmental marks suggest a specific effect on the development (ECETOC guidance). Furthermore, the level of concern is high for changes in time to preputial separation and vaginal opening that are not accounted for by bodyweight. Whether the observed differences in the puberty oneset parameters in this magnitude are of biological significance and due to specific effect should be discussed. There is no consensus on the degree of change that is considered adverse regarding the puberty onset markers. The historical control data provided in RAR includes the study under assessment. However, since there was no dose response in either gender and the slightly delayed onset of puberty at the high dose was only marginally different from the respective control (by 6.4% and 5.2% for females and males, respectively), the level of concern is reduced. When considering the consequences of the observed delay in the puberty onset to the reproductive cycle normality, sexual behavior and fertility of F1 male and female pups when siring the F2 generation, no adverse effects were detected on female nor male parameters (estrous cycle parameters, mating behavior, fertility indices; Table 21).

Table 20 Summary of parameters of puberty onset in F1 pups

Parameter	Dose (ppm)			
	0	50	250	1250
Balanopreputial separation (days, mean ± SD)	41.61 3.08	43.22 2.58	42.33 2.94	43.78* 2.91
BW at balanopreputial separation (mean ± SD)	167.93 23.20	173.33 17.20	169.70 15.88	168.04 16.22
Vaginal opening (days, mean ± SD)	39.07 3.52	40.44 4.72	38.67 4.23	41.56 3.81
BW at vaginal opening (mean ± SD)	127.15 12.54	135.96 14.76	123.30 12.93	130.89 15.84

Table 21 Summary of observations in fertility and reproduction in F1 females and males

Observations	Dose, ppm			
	0	50	250	1250
Number of females used	27	27	27	27
Estrous cycle lenght (days) prior cohabitation, mean ± sd	3.01 ± 0.81	3.78 ± 1.27*	3.69 ± 0.91	3.51 ± 1.21
Number of estrous cycle (in 22 days), mean ± sd	6.52 ± 1.72	5.48 ± 1.70*	5.37 ± 1.01*	5.67 ± 1.44
Number of females cycling normally[a]	10	17	22	15
Number of males housed with female	27	27	27	27
Number of males impregnating female	24	27	27	26
Number of female housed with male	27	27	27	27
Number of female with sperm positive vaginal smear/pregnant	23	26	27	26
Precoital interval (days), mean ± sd[b]	2.09 ± 1.23	2.73 ± 1.22	2.19 ± 1.08	2.38 ± 1.06
No of estrous cycles required (after the day of cohabitation) for mating	27	32	29	29
Male fertility index[c]	88.89	100	100	96.3
Female fertility index[d]	85.19	96.3	96.3	85.19
Mating index[e]	85.19	81.25	93.10	89.66

* $p < 0.05$

[a] Number of females cycling normally was calculated as female having estrous cycle lenght of 4 ± 1 days

[b] Number of days taken to establish sperm positive vaginal smear (mating) from the day of cohabitation

[c] (Number of males impregnating females)/number of males exposed to non-pregnant females) x 100

[d] (Number of pregnant females/number of females exposed to males) x 100

[e] (Number of confirmed copulations (E+)/Number of estrous cycle required to for mating (after the day of cohabitation) x 100

Sensory reactivity (auditory startle, pupil response, air righting reflex, tail pinch reponse) was investigated in 1 male and 1 female pup per litter on lactation day 20 in both generation. No treatment-related effects were observed. Motor activity (total and ambulatory activity) was also examined on day 20 (one pup/gender/dam/dose). Pups were monitored for 3 consecutive 5-minute intervals allowing for examination of both exploratory and acclimation activity levels. Stereotypic activity was calculated by subtracting ambulatory activity from total activity. Total motor activity was significantly elevated during the first 5 minutes at all doses (without a dose response) and during the first 10 minutes at 1250 ppm only in F1 males. In F2 females there was a dose-dependent increase in total motor activity reaching significance at the mid and high doses during the first 5 minutes and in the high dose during the first 10 minutes. In F1 females and in F2 males there was a statistically non-significant increase in total motor activity. During the last 5-minute interval the values were close or comparable to control in both generation and gender. In general, the observed effects could be a sign of delayed development or developmental neuroroxicity. However, the biological significance of these results is difficult to see because there was no clear dose- or time-reponse, and the standard deviation of the means were large indicating great variation in the behaviour between individual animals.

The second 2-generation study in Wistar (KFM-Han) rat (**RAR B.6.6.1/02**) had major deficiencies. The study was performed according to US guidelines for registering pesticides. The following organs were not weighed: epididymis, prostate, seminal vesicle and testis. The number of corpora lutea and implantation sites, and post-implantation losses were not counted. Pup survival data is unreliable because dams cannibalized some of their pups or whole litter. Study reporting regarding statistical analysis is poor.

In the study, the F0 generation was paired twice (employing alternative pairings) to produce F1A and F1B litters, and the F1B litters were used to form the basis of the next generation and also paired twice (employing alternative pairings) to produce F2A and F2B litters.

There were no clinical symptoms or mortality of the parent animals in either generation.

In F0 parent males at 1250 ppm significantly reduced body weights were recorded from week 4 of the prepairing period until day 29 after pairing for breeding F1B litters (last measurement prior necropsy date). Food consumption in F0 generation males was similar in all groups throughout the study.

In F0 parent females at 1250 ppm slightly reduced body weight gain during the prepairing period and continued slightly lower body weights during gestation and lactation periods for breeding of F1A and F1B litters were noted. The differences noted were mostly not significant. Food consumption in F0 generation females was similar in all groups throughout the study.

In F1 parent males at 1250 ppm significantly reduced mean body weights were noted until day 22 of the prepairing period. Thereafter, only insignificant differences were noted until necropsy. Significantly reduced food consumption was observed in Fl parent males at 1250 ppm during the first three weeks and in the 12th week of the prepairing period for breeding F2A litters.

The females of the Fl parent generation displayed significantly reduced body weights at 1250 ppm during the prepairing period, throughout both gestation and lactation periods for breeding F2A and F2B litters and at necropsy. The reduced body weights existed already at the initiation of the prepairing period as consequence of reduced body weight gain during the lactation period as F1B pups. Significantly reduced food consumption was observed in Fl parent females at 1250 ppm during the first three weeks and the twelfth week of the prepairing period as well as during both gestation and lactation periods (breeding F2A and F2B litters).

Hemolytic anemia accompanied by significant increases in splenic weights, erythropoiesis or hemosiderosis in spleen and erythroid hyperplasia in bone marrow and follicular hyperplasia in thyroid were observed at 250 and 1250 ppm in F1 parental animals.

Epididymides, prostate or seminal vesicle were not weighed in this study. There were no treatment-related effects on absolute testes weights (Table 22). There were no histopathological findings in the testes.

Table 22 Testes weight in F0 and F1 parent males

Absolute testes weights (g) mean ± SD	**Dose, ppm**			
Generation	0	50	250	1250
F0	3.74 ± 0.30	3.81 ± 0.34	3.75 ± 0.39	3.69 ± 0.31
F1	3.55 ± 0.88	3.66 ± 0.69	3.85 ± 0.37	3.60 ± 0.28

There was a slight reduction of the number of pups in F2A litters at 1250 ppm (3.6 %) and in F2B litters at 250 ppm (12.7 %) and 1250 ppm (11.0 %) at birth compared to control (Table 23). Similar observation in the litter size was not made for F1A or F1B litters. It was not possible to evaluate pre/postimplantation losses because the number of corpora lutea or implantations sites were not recorded. Pup survival data during lactation is unreliable due to dams cannibalizing some of their pups.

Table 23 Number of pups in F1A, F1B, F2A and F2B litters

Number of pups after first litter check**	**Doses, ppm**			
	0	**50**	**250**	**1250**
F1A litters	321 (11.1)	336 (11.6)	357 (11.9)	323 (11.1)
F1B litters	278 (11.1)	337 (11.2)	313 (10.8)	312 (11.1)
F2A litters	268 (11.2)	319 (12.3)	243 (11.0)	248 (10.8) (-3.6%)
F2B litters	282 (11.8)	283 (11.3)	196 (10.3) (-12.7%)	252 (10.5) (-11.0%)

( )= number per dam
** = immediate whenever possible; could be up to 16 hours (overnight)

Significantly reduced mean body weights were recorded in the pups at 250 ppm of the F1A litters (from day 1 to 21 post partum) and at 1250 ppm of the F1A litters (from day 1 to 21 post partum), of the F1B litters (from day 7 to 21 post partum), of the F2A litters (from day 4 to day 21 post partum), and of the F2B litters (from day 1 to day 21 post partum). No adverse effects were noted in the F1B, F2A and F2B pups at 250 ppm and in any pup generation at 50 ppm.

Changes in pup organ weights (reduced brain, kidney, heart, spleen, liver, thymus) were recorded at 250 and 1250 ppmin both generations and some reductions seemed to be dose-dependent. Some of the observations may be related to the reduced body weights.

In behavioural tests (cliff avoidance, palmar grasp ability, exploratory locomotion) at day 32 p.p. no adverse effects were observed in pups at any dose at any litter in either generation.

10.10.3 Comparison with the CLP criteria

In rat the main findings of possible fertility toxicity concern are the reduced epididymal sperm counts detected in one 2-generation study (RAR B.6.6.1/01, 2003), in the other two 2-generation studies this endpoint was not studied. Reduced sperm counts were not accompanied by any other adverse observations in the male reproductive organs. The terminal relative testis weight, epididymis and cauda epididymis weights were increased however this was due to the reduced terminal body weight since the absolute organ weights were not changed. There were no changes in the histopathology of the male reproductive organs. In other reproductive toxicity studies or in repeated 28- or 90-day toxicity studies there were no relevant observations in the weights of the male reproductive organs. The reduction in the rat sperm number was not reflected as reduced fertility. However this is not uncommon since in rodents effects in fertility may be seen only after drastic reduction in the sperm count. A reduction in rodent sperm number is a relevant finding regarding

fertility in humans (WHO, ECETOC). However, due to the small magnitude in the sperm count reduction, and due to the large variation in the control values (large starndard deviation), the observed reduction in sperm count in the absence of other supporting observations (no change in testes weight, epididymal weight or histopathology) may be considered an isolated finding. According to the ECETOC guidance: "*Changes in sperm parameters in isolation should not be used to judge the reproductive toxicity of a chemical*" and *ILSI (1999): "An isolated change in any one of these endpoints (testicular sperm number, epididymal sperm count, testis weight and morphology) is cause for less concern than a suite of related changes, all internally consistent."* It should also be noted that in the studies on phenmedipham which is structurally and toxicologically related to desmedipham, there were no remarkable findings in testes histopathology in chronic toxicity and repeated dose toxicity studies (CLH report for phenmedipham, Finland). It is therefore concluded that classification for fertility based on observations in total cauda epididymis sperm number is not appropriate.

In the same 2-generation study (RAR B.6.6.1/01, 2003) some observations were made regarding physical developmental landmarks and puberty onset in pups. Considering all of the developmental landmarks together where some effects were detected, no clear pattern can be seen. The reduced body weight correlated with delayed eye opening only in F2 generation but not in F1 generation. The degree of the delay in the eye opening time was small and not accompanied by any other observation in the set of physical development landmarks (unfolding of pinna, tooth eruption, ear opening, hair growth). Therefore the observation of the delayed eye opening is considered an isolated finding and probably a sign of biological variation.

The observed differences in the time of balano preputial separation and vaginal opening compared to the respective controls were small in the magnitude and with no dose response in either gender. Unfortunately this endpoint was only studied in the F1 generation since the F2 pups were killed already after weaning. This endpoint was not examined in the older 2-generation studies, so there are only limited data available. There is no consensus on the degree of an effect considered adverse for this endpoint. However if the magnitude of the difference in the puberty onset time is considered an adverse effect, it cannot be explained by reduced body weight for either gender. If considered a sign of endocrine disruption, there is no data on possible mode of action. The provided historical control data on the puberty onset markers from the study laboratory includes the study under assessement. When considering the consequences of the observed delay in the puberty onset to the reproductive cycle normality, sexual behavior and fertility of F1 male and female pups when siring the F2 generation, it should be noted that there we no delays in any female nor male parameters (estrous cycle parameters, mating behavior, fertility indices). Therefore, if the slight delay in the puberty onset is considered a hazard, homeostasis seemed to have been reestablished at adulthood without long term adverse effect. In conclusion, based on all the available data, it is concluded that the observations on the puberty onset markers are of negligible biological significance and therefore classification for fertility is not appropriate.

In the same 2-generation study (RAR B.6.6.1/01, 2003) some observations were made regarding increased motor activity in the pups. This endpoint is discussed in the section 10.10.6.

There was an observation of a slightly reduced litter size in the F2A and F2B litters at 1250 ppm and at 250 and 1250 ppm, respectively, in the second 2-generation study (RAR B.6.6.1/02, 1986). Data was lacking on some crucial fertility parameters (number of corpora lutea and implantation sites). The level of concern is high if reduction in litter size is observed at a dose that does not cause parental toxicity. In this study the body weights of the F1 females siring the F2A and F2B litters were lower than the controls (by 7.5%) already at the start of the treatment due to reduced body weight gain as F1B pups during lactation period. This difference remained nearly unchanged throughout the study. Food consumption of the F1 females was also significantly reduced at 1250 ppm during the first three weeks and the twelfth week of the prepairing period as well as during both gestation and lactation periods. Reduced body weight and reduced food consumption were recorded for F1 males during some periods of prepairing for breeding F2A litters. Due to the obvious maternal toxicity at 1250 ppm the level of concern is reduced for smaller litter size. It is therefore concluded that classification for fertility is not appropriate.

10.10.4 Adverse effects on development

Table 24: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results			Reference
		Maternal NOAEL	Developmental NOAEL	Effects	
Teratogenicity study OECD 414 GLP Wistar rat 24 females/group	Desmedipham 99.8 % 0, 10, 100, 500 mg/kg bw/day Oral intubation GD 6-15	100 mg/kg bw/day	10 mg/kg bw/day	Maternal: decrease in the body weight gain at 500 mg/kg bw/day on GD 6-10 (-15%) and GD 11-15 (-5%) Developmental: increased incidence of infarct of liver, bipartite ossification of sternebra at 100 and 500 mg/kg bw/day, increased incidence of incomplete ossification of interparietal bone and increased incidence of absent sternabra at 500 mg/kg bw/day, increased incidence of hemorrhagic kidney at all doses	2001 RAR B.6.6.2/01 M- 493963-01-1 Key study
Embryotoxicity/teratogenicity study Equivalent to OECD 414 (statistically significant results were not reported in summary tables except for sex ratio of live foetuses) GLP Wistar rat (KFM-HAN, outbred, SPF-quality) 25 females/group	Desmedipham 97.8 % 0, 10, 100, 1000 mg/kg bw/day Oral intubation GD 6-15	10 mg/kg bw/day	10 mg/kg bw/day	Maternal: reduced body weight and corrected body weight gain, and reduced food consumption at 1000 mg/kg bw/day and decreased corrected body weight gain at 100 mg/kg bw/day Developmental: increased number of foetuses with supernumerary rib at 100 mg/kg bw/day Malformations: at 100 mg/kg: 1 fetus with agnathia (inferior) and open eyes; 1 fetus with omphalocele; at 1000 mg/kg: 6 fetuses with palatoschisis and slight micrognathia (inferior) and 1 fetus with palatoschisis, distinct micrognathia and dysplastic tail in 1 litter; 1 runt fetus	1985a RAR B.6.6.2/02 M-146758-01-1 Key study
Embryotoxicity/teratogenicity study Equivalent to OECD 414 (statistically significant	Desmedipham 98.3 % 0, 10, 100, 500 mg/kg	7 mg/kg bw/day (3.7 % MetHb)	70 mg/kg bw/day	Maternal: increased methemoglobinemia at 70 and 350 mg/kg bw/day Developmental: decreased	1985b RAR B.6.6.2/03

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**			**Reference**
		Maternal NOAEL	**Developmental NOAEL**	**Effects**	
results were not marked in summary tables. At the end of the dosing period blood samples were collected and Heinz bodies and methemoglobin were determined) GLP Wistar rat (KFM-HAN, outbred, SPF-quality) 35 females/group	bw/day (corrected 0, 7, 70, 350 mg/kg bw/day because achieved test material concentrations were 70% and 77% of the target value at the dose level of 10 mg/kg bw at the study initiation and termination) Oral intubation GD 6-15			fetal body weight and increased incompletely ossified sternebrae and absent ossification of phalangeal nuclei, calcanea and cervical vertebrae at 350 mg/kg bw/day 1 fetus with micrognathia at 350 mg/kg bw/day, 1 runt fetus at at 70 mg/kg bw/day, 1 fetus with generalized hydrops at at 70 mg/kg bw/day	M-146758-01-1 Key study
Preliminary teratogenicity study Range finding study, non-guideline GLP Sprague-Dawley rat 8-9 mated females/group	Desmedipham 98% 0, 125, 250, 500, 1000 mg/kg bw/day Oral intubation GD 6-15	500 mg/kg bw/day	250 mg/kg bw/day	Maternal: decreased body weight and food consumption at 1000 mg/kg bw/day Developmental: decreased fetal body weight at 500 and 1000 mg/kg bw/day	1990 RAR B.6.2.2/04 M-146989-01-1 Supplemental study
Teratogenicity study OECD 414 (limitations in statistical analyses) GLP Sprague-Dawley rat 25 mated females/group	Desmedipham 98% 0, 60, 250, 1000 mg/kg bw/day Oral gavage GD 6-16	60 mg/kg bw/day	250 mg/kg bw/day	Maternal: increased spleen weight and discoloured urine at 250 and 1000 mg/kg bw/day Developmetal: increased incidence of subcutaneous haemorrhage at 250 and 1000 mg/kg bw/day. Fetal weight reductions, delayed ossifications at 1000 mg/kg bw/day. At 1000 mg/kg bw/day: 3 foetuses in the same litter with cleft palate; 5 fetuses with cardiovascular malformations/abnormalitie	1991 RAR B.6.6.2/05 M-146990-01-1 Key study

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**			**Reference**
		Maternal NOAEL	**Developmental NOAEL**	**Effects**	
				s (2 fetuses with interventricular septal defect, 2 fetuses with partial duplication of inferior vena cava, 1 fetus with multiple malformations interventricular septal defect, hydronephrosis, testes not fully descended).	
Embryotoxicity/teratogenicity study OECD 414 (long dosing period, limited statistical analysis, limitations in the reporting of the results) GLP (but minor exeptions, however not clearly identified in the study report) Chinchilla hybrid rabbit 16 mated females/grpup	Desmedipham 97.8 % 0, 50, 150, 450 mg/kg bw/day Oral gavage GD 6-27	< 50 mg/kg bw/day	< 50 mg/kg bw/day	Maternal: decreased body weight gain at all doses Developmental: decreased fetal body weight at all doses	1984 RAR B.6.6.2/06 M-146731-01-1 Key study
Preliminary teratogenicity study Tolerance and range finding study, non-guideline GLP New Zealand White rabbit 3 unmated females/group in tolerance study/ 8 mated femals/group in range finding study	Desmedipham 98 % 125/500, 250, 1000 mg/kg bw/day/dosed for 2-8 days, rested for 2-7 days 0, 40, 120, 360 mg/kg bw/day/ GD6-18	< 40 mg/kg bw/day	120 mg/kg bw/day	Maternal: reduced body weight gain at all doses Developmental: decreased fetal body weight and increased post-implantation losses at 120 mg/kg bw/day	1991a RAR B.6.6.2/07 M-146987-01-1 Supplemental
Teratogenicity study OECD 414 (limited statistical reporting) GLP New Zealand White rabbit 16 mated females/group	Desmedipham 98 % 0, 30, 90, 270 mg/kg bw/day Oral gavage GD 6-18	30 mg/kg bw/day	30 mg/kg bw/day	Maternal: decreased body weight and food consumption at 270 mg/kg bw/day, increased spleen weight at 90 and 270 mg/kg bw/day Developmental: increased percentages of early embryonic death at 90 and 270 mg/kg bw/day, increase in slight caudal pelvis shift at 270 mg/kg bw/day	1991b RAR B.6.6.2/08 M-146987-01-1 Key study

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

Developmental toxicity of desmedipham was investigated in the following studies: in two range-finding studies in rat and rabbit, in four teratogenicity/embryotoxicity studies in rat and in two teratogenicity/embryotoxicity studies in rabbit. All of the studies were conducted according to the old version (1981) of OECD 414 test guideline or equivalent and therefore there are deviations compared to the new test guideline and also deficiencies in reporting of the results and statistics. The treatment period covered the organogenesis except for one rabbit study where the dosing period was until GD 27. Only relevant studies for classification are described briefly here and further details are given in the RAR including one additional limit test in rat (dermal application) which is not summarised here because it is not considered relevant for classification.

In the newest teratogenicity study in Wistar rat (**RAR B.6.6.2/01, 2001**) not assessed before in the PPP programme no maternal toxicity was observed however there was a slight decrease in the dam body weight gain at the highest dose of 500 mg/kg bw/day at gestation days 6-10 (-15%) and 11-15 (-5%). Feed consumption in the treated groups were comparable to control group throughout the gestation period. No treatment-related clinical signs or mortality were observed during the study. No difference in the pregnancy data of control and treated groups was seen. Prenatal parameters, number of corpora lutea, implantation rates, live and dead foetuses and resorptions were not affected by treatment. The mean litter size, litter weights, average fetal weights and mean number of male and females per litter were not affected by treatment.

There was an increased fetal incidence of infarct of liver at 100 and 500 mg/kg bw/day, a finding which was statistically significant at fetal level but not at litter level (5/5, 6/6, 19/10, 17/8 no. of fetuses/no. of litter at 0, 10, 100 and 500 mg/kg bw/day, respectively). The level of concern is high for fetal liver infarct observation and in the historical control data of the performing laboratory (9 studies from 1997-2002, 849 fetuses, 198 litters, Wistar rat) there were no incidences of liver infarct in fetuses. However in the present study there was no dose response, and there was a background level of liver infarct incidences in the control. For these reasons the level of concern for this observation is reduced.

In the fetuses statistically significantly increased incidence of haemorrhagic kidney was observed in all treatment groups but not in the control group, but there was no dose reponse either at the fetal or the litter level. The observed incidences were above the historical control data of the performing laboratory (9 studies during 1996-2002, 10/849, 1.18% fetuses, in 6/198, 3.03% litters, RAR). The significance of this finding is unclear and it cannot clearly be linked to the desmedipham treatment. There were statistically non-significant hemorrhages also in other organs (thymus, lung) in all treatment and control groups.

Fetal skeletal observations of ossification variations revealed statistically significant difference in incomplete ossification of interparietal bone of fetal and litter incidence at 500 mg/kg bw and of fetal incidence of bipartite occification in sternebrae at 100 and 500 mg/kg bw/day. The incidence of absent sternebra was increased at 500 mg/kg bw/day but with similar malformations also in the control group at a lower level. Since these were observed in the absence of maternal toxicity and some of the effects were dose-dependent, this may be considered a treatment-related effect and therefore raise a concern for developmental toxicity of desmedipham.

In an embryotoxicity/teratogenicity key study in Wistar KFM-Han rats (**RAR B.6.6.2/02, 1985a**) maternal toxicity was evident at 1000 mg/kg bw/day based on reduced corrected body weight gain at 1000 (-53%) and reduced food consumption (12.4%). There was slightly reduced corrected body weight gain also at 100 mg/kg bw/day (-17%) but this was statistically non-significant. There was no mortality or clinical signs in the dams during the study.

Mean fetal body weight was significantly reduced at 1000 mg/kg (by 12.5%) compared to the control group. External investigation on fetuses revealed the following malformations: 1 fetus with agnathia and bilateral open eye at 100 mg/kg bw/day; 7 fetuses in the same litter with palatoschisis and with slight (6 fetuses) or distinct (1 fetus) microagnathia at 1000 mg/kg bw/day, the latter fetus had also a dysplastic tail. In addition at 100 mg/kg bw/day 1 fetus/litter had omphalocoele and another fetus in a different litter was a

runt. All of these malformations are of high concern and may be related to desmedipham treatment. The applicant has submitted historical control data from the performing laboratory on Wistar rats. The data is from prenatal developmental toxicity studies conducted during 1992-1995 (RAR B.6.6.2/02 was conducted in 1984). Other details are missing. According to RAR it is not clear if the malformations in the HCD are categorized as the same malformation as those observed in this study. According to this historical control data the incidence of microagnathia was 0-0.3% in foetuses and 0-4.2% in litters in studies conducted during 1992-1995 in the laboratory.

In the skeletal investigations of the fetuses abnormalities included absent sternebrae (nos. 5, 6), abnormally shaped sternebrae (nos. 4, 5), dumbbell shaped thoracic vertebral body (nos. 10, 11, 12) longitudinally split sternebrae (nos. 3-5) and wavy ribs (nos 11-13). The proportion of fetuses with these skeletal abnormalities was higher at 10 and 1000 mg/kg bw/day compared with the control group. Although the abnormal or absent sternebra are of high concern, the level of concern is reduced since there was no dose response and similar observations were made also in the control group.

The number of fetuses with supernumerary ribs was increased at 100 and 1000 mg/kg bw/day (doubled) compared with the controls. However due to the deficiencies in reporting the distribution of the affected fetuses in each litter is unclear. These observations can be explained by maternal toxicity or they are incidental effects.

There was an increase in the incidence of incompletely ossified cranial os occipital, sternebra, thoracic vertebra and phalangeal nuclei at 1000 mg/kg bw/day. All of these findings can be explained by the reduced fetal body weight at this dose reflecting defective maturation caused by maternal toxicity.

Because of the mandibular malformations observed in the aforementioned study (**RAR B.6.6.2/02, 1985a)**, a second study (**RAR B.6.6.2/03, 1985b**) was performed using the same rat strain and the same procedures with the exception of reducing the highest dose from 1000 to 500 mg/kg bw/day and increament of the number of females from 25 to 35 per group. In addition to the parameters usually examined in a embryotoxicity/teratogenicity study, some hematology measurements (methemoglobin, Heinz bodies) on day 16 were performed because of the toxic hemolytic effects of desmedipham. Due to the low achieved nominal concentrations of desmedipham (70% and 77%), it was suggested to use corrected doses of 7, 70 and 350 mg/kg bw /day.

Maternal toxicity was indicated by significantly reduced body weight and reduced corrected body weight gain and reduced food consumption at 500 mg/kg bw/day during the treatment period. Elevation of methemoglobin concentration was found in all dosed groups which was significant at 100 mg/kg bw/day (3.7%) and at 500 mg/kg bw/day (9.3%). Significant increase in Heinz body count was increased at 500 mg/kg bw/day. There was no mortality or clinical signs during the study.

Mean fetal body weight was slightly but significantly reduced at 500 mg/kg bw/day (10.6%). External investigation on foetuses revealed the following malformations: one runt with absent 5^{th} and 6^{th} sternebra and another fetus in different litter with generalized hydropsis at 100 mg/kg bw/day; one fetus with inferior micrognathia at 500 mg/kg bw/day. Since this malformation (micrognathia) was observed also in the previous study, this raises the concern for developmental toxicity.

In skeletal investigations reduced state of skeletal ossification was observed manifested as an increase in incompletely ossified sternebrae and absent ossification of phalangeal nuclei, calcanea and cervical vertebrae in fetuses at 500 mg/kg bw/day. These skeletal observations can be attributed to the maternal toxicity and consequently reduced fetal weight at this dose level.

In a rat teratogenicity range finding study in Sprague-Dawley rats (**RAR B.6.6.2/04, 1990**) maternal toxicity was clearly demonstrated as reduced maternal body weight (35%), reduced food consumption (20%) at 1000 mg/kg bw/day. Mean fetal body weight was reduced at all doses (by 5%, 5%, 16% and 25% at 125, 250, 500 and 1000 mg/kg bw/day, respectively). The incidences of postimplantation losses were 3%, 7%, 3%, 7% and 5% at 0, 125, 250, 500 and 1000 mg/kg bw/day, respectively. All of the other reproduction parameters (pregnancy frequency, corpora lutea, implantations, dead and live fetuses) were similar in all groups. Based on the results of this study it was considered that suitable dose levels for a subsequent teratogenicity study would be 0, 60, 250 and 1000 mg/kg bw/day.

In the main rat teratogenicity study in Sprague-Dawley rats (**RAR B.6.6.2/05, 1991**) maternal toxicity was clearly demonstrated at 1000 mg/kg bw/day as reduced food consumption (8%) and reduced body weight gain (29%). Most animals (18/25) receiving 1000 mg/kg bw/day showed discoloured urine on one or more occasions, and three animals at 250 mg/kg bw/day, indicating hemolytic anemia. During the course of the necropsies, enlargement of the spleen was noted at 1000 mg/kg bw/day. The spleen weights of all pregnant animals were therefore recorded. The mean spleen weights were significantly increased at 250 mg/kg bw/day (19 %) and at 1000 mg/kg bw/day (81%) compared with control.

There was an increased incidence of post-implantation loss in the treated groups but after a through analysis of all of the reproductive toxicity studies (no post-implantation losses) and taking account of the historical control data of the performing laboratory (12 studies during 1989-1992, post-implantation loss range 8/364 (2.2%) – 21/262 (8.0%), Sprague Dawley, RAR) it was concluded that the observed incidences were comparable with the those seen in historical controls.

Mean fetal body weight was reduced at 1000 mg/kg bw/day being 20.5% lower than in control. External investigation on fetuses revealed the following malformations: 7 fetuses from the same litter with slight kyphosis of cervico/thoracic spine, dorsal-ventral curvature of sternum, and with shortened ribs and misshapen clavicles at 250 mg/kg bw/day; 3 fetuses in the same litter with cleft palate at 1000 mg/kg bw/day. There was an increase in retardation of ossification of occipital bone and the scapulae at 1000 mg/kg bw/day. Visceral investigations revealed cardiovascular malformations/abnormalities in 5 fetuses in 3 litters at 1000 mg/kg bw/day: one fetus with multiple malformations (interventricular septal detect, hydronephrosis, testes not fully descended) and in the same litter another fetus had an intervenricular septal defect; one fetus in different litter with interventricular septal defect and right atrium enlarged and right ventricle reduced; two fetuses in the same litter with partial duplication of inferior vena cava.

Of the malformations kyphosis has been seen in the performing laboratory before and usually affects many fetuses in the litter, therefore it is not considered a treatment-related effect. Cleft palate is a rare finding and the level of concern of this malformation is high. However all of the fetuses affected were in the same litter of a dam treated with a maternally toxic dose. Cardiovascular malformations (interventricular septal defect, partial duplication of inferior vena cava) were also observed only at maternally toxic dose but in different litters. The etiology of these effects could be attributed to a genetic background. However since it cannot be exluded that these observations are treatment-related, they cause additional concern for developmental toxicity.

In an embryotoxicity/teratogenicity study in Chinchilla hybrid rabbits (**RAR 6.6.2/06, 1984**) the dosing period was longer than normal, until gestation day 27. There were deficiencies in the study reporting (limited statistical analysis, poor reporting of clinical observations and necropsy findings). Maternal toxicity was observed at 450 mg/kg bw/day as reduced mean body weight gain (8.0% decrease compared to controls over days 6-28), reduced corrected body weight gain (-2.6, -3.1, -4.3 and -7.1% of the mean weights on day 6 at 0, 50, 150 and 450 mg/kg bw/day, respectively) and reduced food consumption during the treatment period (25.6% decrease compared to controls). At the low (50 mg/kg bw/day) and mid dose (150 mg/kg bw/day) there was transiently reduced body weight gain of dams during days 2 - 3 and during days 2-8, respectively, whereafter the body weights were comparable to the control. One female at 150 mg/kg bw/day aborted all foetuses on day 27 and two females at 450 mg/kg bw/day aborted between days 27 and 28. One of these high dose dams aborted all foetuses. The females with total abortions at 150 and 450 mg/kg bw/day were excluded from calculations of body weight, corrected body weight, food consumption and reproduction data. Statistical significances of body weights, food consumption and reproduction data were not reported.

Despite of unclear reporting of the number of implantations, embryonic and fetal resporptions, abortions and live and dead fetuses at 150 and 450 mg/kg bw/day there was a general increase in postimplantation losses at 450 mg/kg bw/day. The mean fetal body weight was reduced by 10.5%, 12.7 and 31.6% at 50, 150 and 450 mg/kg bw/day, respectively. There were no malformations in fetuses at any dose level. Skeletal investigations revealed statistically significant increament in the number of non-ossified phalangeal nuclei in the fore and hindlimbs in fetuses, and at litter basis mainly at the highest dose. It is concluded that the postimplantation losses and the reduced fetal body weight may have been caused by primary maternal toxicity at 450 mg/kg bw/day, and that the skeletal findings can be attributed to the reduced fetal weight.

In a teratogenicity tolerance and range finding study in New Zealand White rabbits (**RAR 6.6.2/07, 1991a**), unmated female rabbits were dosed in the first phase with varying doses of desmedipham (125/250, 500, 1000 mg/kg bw/day) for varying periods of time. There was a reduction in the body weight and food consumption at doses of 250, 500 and 1000 mg/kg bw/day. Based on this result, a range-finding study with mated female rabbits was carried out using desmedipham 40, 120 or 360 mg/kg bw/day. Also in this study body weights at were reduced all doses and food consumption at 360 mg/kg bw/day. At this dose only four dams out of seven reached day 22 of gestation and others aborted their litter. It is concluded that desmedipham was embryotoxic by causing post implantation losses at a maternally toxic dose of 360 mg/kg bw/day.

In a main teratogenicity study in New Zealand White rabbits (**RAR 6.6.2/08, 1991b)** the highest desmedipham dose was lowered to 270 mg/kg bw/day. Maternal toxicity was clearly observed at 270 mg/kg bw/day as significant reductions in mean body weight gain (29%) and reduced food consumption (49%). The spleen was enlarged at the highest dose, therefore the spleen was weighed at all doses. The absolute spleen weight increased by 19% at 90 mg/kg bw/day and by 17% at 270 mg/kg bw/day. Reduced fecal excretion was observed in some dams in each group but at the highest dose this was observed in 14/15 of dams.

Desmedipham increased the percentage of early embryonic deaths at 90 and 270 mg/kg bw/day. The mean fetal body weight was reduced by 7% at 270 mg/kg bw/day. There were various abnormalities and variants in the fetuses at 90 mg/kg bw/day but these were considered not to indicate an adverse effect of treatment with desmedipham. Skeletal investigations revealed slightly retarded ossification at 270 mg/kg bw/day. This effect was probably related to treatment and may have been secondary to the maternal toxicity. There was an incidence of slight but dose-dependent caudal pelvic shift of 1.2%, 1.6%, 9.8%, 12.8% at 0, 30, 90 and 270 mg/kg bw/day, respectively. It is concluded that the incidences of early embryonic deaths and the increased incidences of caudal pelvic shift cause additional concern for developmental toxicity.

10.10.6 Comparison with the CLP criteria

According to the classification criteria, category 1A should be allocated to substances known to produce adverse effects on development mainly based on human evidence. There is no human data on desmedipham on developmental effects, therefore **classification as Repr. 1A is not appropriate for developmental toxicity**.

In rat the main findings of possible developmental toxicity concern are the facial malformations palatoschisis with slight or distinct micrognathia in two studies in Wistar rat (7 fetuses in one litter in study 1/ 1 fetus in one litter in study 2) and cleft palate in a study in Sprague-Dawley rat (3 fetuses in one litter). Palatoschisis is a synonym for cleft palate. Cleft palate is a rare finding and the level of concern for this malformation is high. Whether micrognathia and cleft palate are separate malformations with distinct etiology is not clear. Recent research suggest that there might be a causative relationship with micrognathia inducing cleft palate in humans and animals (Price et al 2016). Cleft palate/palatoschisis was observed in two separate studies with two different rat strains (Wistar, Sprague-Dawley) but at maternally toxic dose and the affected foetuses were in the same litter in both studies. Micrognathia was observed in two separate studies but in the same Wistar rat strain. It is not known that maternal toxicity could induce cleft palate or micrognathia in rat. There was also one fetus at maternally non-toxic dose with agnathia and open eyes in the Wistar rat study. It should be noted that in the teratogenicity toxicity studies with phenmedipham there was one fetus with brachygnathia in a control group and one fetus in a high dose group in a teratogenicity range-finding study (CLH report of phenmedipham, Finland). According to the historical control data of Wistar rat strain the incidence of micrognathia was 0-0.3% in foetuses and 0-4.2% in litters in studies conducted during 1992-1995 suggesting a genetic etiology of some level. The studies under assessment were however carried out a decade before the HCD studies. In conclusion, taking all the evidence into account, the level of concern is reduced for the incidence of both cleft palate and micrognathia, therefore classification as Repr. 1B is not appropriate since sufficiently convincing evidence is lacking. However since there was one fetus with agnathia at maternally non-toxic dose and since it cannot be exluded that these observations are treatment-related, and thus some uncertainty remains, classification as **Repr 2 for development is considered approriate**.

In the same teratogenicity study in Sprague-Dawley rat discussed above, occurrence of cardiovascular malformations (interventricular septal defect and partial duplication of inferior vena cava, 5 affected foetuses in 3 litters) were observed at maternally toxic dose. These findings are relevant for humans and the etiology of these could be attributed to a genetic background. However, since it cannot be excluded that these malformations are treatment-related, **Repr 2 development is considered approriate**.

In the newest teratogenicity study in Wistar rat skeletal observations of ossification variations were made at the mid (100 mg/kg bw/day) and high dose (500 mg/kg bw/day) of desmedipham. The incidence of incomplete ossification of interparietal bone was increased at both fetus and litter level but there was no dose-response. Bipartite ossification of sternebra was increased at fetal but not at litter level. This observation is considered a variation of low to moderate concern. The incidence of absent sternebra was increased at both fetus and litter level with no dose-response and with occurrence also in the control group. The level of concern of absent sternebra malformation is high. There was no maternal toxicity at the mid and high dose judged by the absence of clinical observations, reduced body weight gain or reduced food consumption. Hematological measurements were not made and the spleen weights were not recorded however in some dams atrophy in the spleen was recorded. However based on some other reproductive toxicity studies and the repeated dose toxicity studies on desmedipham in rat hematological effects (methemoglobinemia) can be assumed at least at the highest dose level. Taking all information into account including the fact that there was a baseline incidence level of absent sternabra also in the control group it is concluded that the level of concern is reduced for the skeletal effects **and no classification is considered appropriate.**

In the 2-generation study (RAR B.6.6.1/01, 2003) under section 10.10.2 some observations were made regarding increased motor activity in the F1 and F2 pups tested on day 20 p.p. The statistically significantly increased total motor activity in F1 males (with no reduced body weight) and in F2 females (with reduced body weight) during the first 5-10 minutes of the test and the non-significantly increased motor activity in F1 females (with no reduced body weight) and in F2 males (with reduced body weight) were observed. There was no clear dose- or time-reponse, and the standard deviation of the means were large indicating huge variation between individual animals. The observations in the motor activity cannot consistently be explained by delayed development based on the reduced body weight. There are no indications of desmedipham being neurotoxic. No effects were seen in behavioural and neuromuscular tests in the second 2-generation study (RAR B.6.6.1/02, 1986). However, since the underlying reason for the increased motor activity cannot exclusively be explained by a general delay in development, the results raise an additional concern for developmental toxicity.

In New Zealand White rabbit desmedipham caused early embryonic death at a dose of 90 mg/kg bw/day and a dose-dependent increase in the slight caudal pelvic shift. However, it is not clear whether this dose was maternally non-toxic since at this dose there was a non-significant enlargement of the spleen indicating haemolytic anemia of some degree. This finding was accompanied by a reduced food consumption (9%) and reduced fecal output in some dams at this dose. It is concluded that the incidences of early embryonic deaths at 90 mg/kg bw/day and the dose-dependently increased incidences of caudal pelvic shift cause additional concern for developmental toxicity.

10.10.7 Adverse effects on or via lactation

There are no data available for effects on or via lactation for phenmedipham.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

10.10.9 Comparison with the CLP criteria

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Based on developmental toxicity effects seen in reproductive toxicity studies in rats and rabbits, classification of desmedipham for **Repr. 2, H361d** (Suspected of damaging the unborn child) is proposed. No classification for fertility and effects on or via lactation is proposed.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

Three 2-generation studies are available. One of them, B.6.6.1/03, was considered unreliable by the DS and excluded from the assessment due to high pup mortality unrelated to treatment and deficient reporting.

The DS discussed several findings related to fertility but did not consider them sufficient for classification for the following reasons:

- Reduced epididymal sperm counts in study B.6.6.1/01, due to the relatively small magnitude and lack of other adverse effects in male reproductive organs
- Delayed puberty onset in study B.6.6.1/01, due to the relatively small magnitude and lack of reproductive effects in F1 adults
- Reduced litter size in study B.6.6.1/02, due to concurrent maternal toxicity

Development

Six PNDT studies are available, four in rats and two in rabbits. The DS proposed classification in Category 2 for adverse effects on development based on the following findings:

- Cleft palate in the rat studies B.6.6.2/02 and /05
- Micrognathia in the rat studies B.6.6.2/02 and /03
- Interventricular septal defect and partial duplication of inferior vena cava in the rat study B.6.6.2/05

According to the DS, classification is further supported by increased incidence of early embryonic death and slight caudal pelvic shift in the rabbit PNDT study (B.6.6.2/08) and by increased motor activity in pups on PND 20 in the 2-generation study (B.6.6.1/01).

The DS did not consider the findings sufficient for Category 1B because the malformations were observed mostly at maternally toxic doses and were usually limited to one litter per

study.

Lactation

The DS did not evaluate the potential of desmedipham to induce adverse effects on or via lactation due to lack of data.

Comments received during public consultation

Comments were received from 4 MSCAs and 1 Industry association.

Two MSCAs supported the DS's proposal of Repr. 2; H361d. The other 2 MSCAs agreed with Repr. 2 for development but additionally proposed a Category 2 classification for fertility on the basis of reduced epididymal sperm count in study B.6.6.1/01. The DS replied that the magnitude of the change has to be taken into account as well as the large standard deviations and also that there was no effect on sperm production (no change in the homogenisation-resistant testicular spermatid number). They hypothesised that the effect, if treatment-related, could be e.g. due to reduced sperm transit time through the epididymis like in the cases of sibutramine or diethylstilboestrol (Borges *et al.*, 2013; Fernandez *et al.*, 2008). The DS noted that reduced sperm transit time may adversely affect sperm maturation and that the fertility classification might be a borderline case.

The industry association disagreed with the proposed developmental classification, bringing forward the following arguments in support of no classification:

- Rat PNDT study B.6.6.2/02:
    - Palatoschisis and slight micrognathia occurred only in 1 litter.
    - Agnathia was observed only at the mid-dose but not at the high dose, so it is unlikely to be a treatment-related effect.
    - The malformations observed in this study had been noted in the HCD of that particular rat strain. The maternal animals had increased MetHb concentrations (investigated in B.6.6.2/03) and maternal hypoxia might have enhanced the overall frequency of spontaneous malformations.
- Rat PNDT study B.6.6.2/05:
    - The top dose was most likely above the MTD as indicated by reduced food consumption and body weight gain, discoloration of the urine and increased spleen weight. In addition, other studies reported distinct increases in MetHb starting from relatively low dose levels.
    - Cleft palate and interventricular septal defect may be secondary to maternal hypoxia (Webster and Abela, 2007).
    - The incidences of the interventricular septal defect were within a published HCD range (Lang, 1993).
- Rabbit PNDT study B.6.6.2/06:
    - The increase in post-implantation loss was mainly caused by maternal toxicity (body weight gain reduction by 51 % and increased number of abortions at the

top dose). No increase in post-implantation loss was observed in the rabbit study B.6.6.2/08 where the maternal toxicity at the top dose was less severe.

In their reply, the DS acknowledged the possibility that malformations may be secondary to hypoxia or occur spontaneously due to a genetic background. On the other hand, they pointed out that cleft palate was observed in two separate studies and in two different rat strains and malformations of the jaw were observed in two studies in one strain. In addition, these malformations were not observed in concurrent controls. As to the rabbit studies, an increase in early embryonic deaths and caudal pelvic shift was observed also at a dose that was not maternally toxic (90 mg/kg bw/d in study B.6.6.2/08). In view of these uncertainties, the DS maintained that classification in Category 2 was appropriate.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

Three generational studies are available for desmedipham. All of them were conducted under GLP. The study B.6.6.1/01 was performed according to the latest version of the OECD TG 416 from 2001. The other two studies (B.6.6.1/02 and /03) were older and generally comply with the older version of the OECD TG 416 from 1983. The two older studies did not investigate sperm parameters and puberty onset.

2-generation study B.6.6.1/01

Although the study report contains a GLP compliance statement, the fact that the initial report contained puberty onset data for F2 generation that was actually sacrificed on PND 21 led the Co-Rapporteur Member State to request a GLP study audit. The GLP audit was conducted 14 years after the in-life phase of the study. Although the audit did not identify major deficiencies constituting non-compliance, some of its findings raise doubts about the overall quality of reporting (e.g., inconsistencies in raw data; reporting of data on a non-existent pup in another study). In addition, RAC previously considered a study on another substance conducted by this laboratory unreliable (PNDT study by Anon., 1999b, in the RAC opinion on mancozeb, 2019). Overall, the reliability of the study B.6.6.1/01 is considered sufficient for inclusion in the assessment, but certain doubts about proficiency of the laboratory and accuracy of reporting remain.

Parental toxicity at the top dose level of 1 250 ppm, corresponding to approx. 140/190 mg/kg bw/d (m/f, P-generation, grand mean), was limited to modest body weight reductions (up to 9 % and 13 % in P and F1 parental females, respectively) and indications of haematotoxicity (increased hemosiderin pigment in the spleen, increased splenic haematopoiesis). The rationale for the top dose selection is not provided in the study report. RAC notes that doses around 4 000 ppm induced significant haematotoxicity (Hb reduction by ca. 15 %, MetHb levels of ca. 10 %, pallor) and marked body weight reduction (by ca. 20 %) in 90-day rat studies (B.6.3.2/02, /04). Therefore, the top dose selection is considered acceptable.

The study reported two findings potentially relevant for fertility classification: (1) reduced epididymal sperm count in P and F1 males; and (2) delayed puberty onset.

The data on sperm parameters (see the table below) show a statistically significant reduction in epididymal sperm count in both generations at 1 250 ppm (by 10 %) and in the F1 generation also at 250 ppm (by 8 %). Testicular sperm count was not appreciably reduced nor was there a biologically significant effect on sperm motility or morphology. A reduction in

epididymal sperm count may generally indicate adverse changes (e.g. reduced epididymal sperm transit time leading to impaired sperm maturation). However, the concern is reduced by the relatively low magnitude of the decrease, the fact that the reduction was smaller than the difference between generations (i.e. the difference is still within normal variability) and by the lack of biologically significant changes in other sperm parameters (motility, morphology, testicular sperm count) or in male reproductive organs (histopathology, weight).

Sperm parameters in study B.6.6.1/01				
Dose (ppm)	**0**	**50**	**250**	**1 250**
Dose (mg/kg bw/d)	0	5	28	137
F0				
Homogenisation-resistant testicular spermatid head count (× 10^6/g); ± SD	150.9 (± 6.7)	146.4** (± 5.7)	146.0** (± 4.1)	146.1** (± 5.2)
Cauda epididymal sperm count (× 10^6/g)	1 180 (± 171)	1 145 (± 113)	1 164 (± 130)	1 058* (± 114)
Sperm motility (% motile)	91.5 (± 1.1)	92.0 (± 0.9)	91.5 (± 0.9)	90.5** (± 0.9)
Sperm morphology (% abnormal)	6.0 (± 2.8)	6.0 (± 3.6)	8.9 (± 8.1)	6.2 (± 2.0)
F1				
Homogenisation-resistant testicular spermatid head count (× 10^6/g)	124.2 (± 11.9)	125.7 (± 17.8)	124.5 (± 14.1)	123.9 (± 22.6)
Cauda epididymal sperm count (× 10^6/g)	995 (± 126)	950 (± 86)	914** (± 66)	895** (± 115)
Sperm motility (% motile)	92.0 (± 0.9)	90.9** (± 0.8)	91.3 (± 2.0)	90.4** (± 0.7)
Sperm morphology (% abnormal)	6.7 (± 2.8)	8.5 (± 3.5)	7.6 (± 3.0)	8.4 (± 3.5)

Statistically significant difference from control: *, p ≤ 0.05; **, p ≤ 0.01 (if Bartlett's test was not significant, parametric ANOVA followed by Dunnett's test; if Bartlett's test was significant, Student's t-test)

Preputial separation (PS) and vaginal opening (VO) were delayed by 2.2 and 2.5 days respectively at the top dose (see the table below). Anogenital distance was not measured in this study. The concern is somewhat reduced by the lack of statistical significance and lack of a dose-response relationship for the day of vaginal opening and the magnitude of the delay in preputial separation being at the border of normal variability for this endpoint.

Puberty onset in study B.6.6.1/01					
Dose (ppm)	**0**	**50**	**250**	**1 250**	**HCD[a]**
Preputial separation					
Day of PS; ± SD	41.6 (± 3.1)	43.2 (± 2.6)	42.3 (± 2.9)	43.8* (± 2.9)	37.5–40.9
Bw on the day of PS (g)	168 (± 23)	173 (± 17)	170 (± 16)	168 (± 16)	
Bw on PND 21 (g)	42.3	44.4*	43.8	43.8	
Vaginal opening					

Day of VO	39.1 (± 3.5)	40.4 (± 4.7)	38.7 (± 4.2)	41.6 (± 3.8)	37.9–41.4
Bw on the day of VO (g)	127 (± 13)	136 (± 15)	123 (± 13)	131 (± 16)	
Bw on PND 21 (g)	40.3	44.2**	42.6*	42.3	

Statistically significant difference from control: *, p ≤ 0.05; **, p ≤ 0.01 (if Bartlett's test was not significant, parametric ANOVA followed by Dunnett's test; if Bartlett's test was significant, Student's t-test)

[a] 3 studies conducted by the same laboratory within 2 years from the current study

2-generation study B.6.6.1/02

Two litters per generation were produced in this study, with the F1B litter being selected to form F1 parents. Pup survival data are of limited reliability due to several dams cannibalising their pups.

Parental toxicity at the top dose of 1 250 ppm (approx. 90/140 mg/kg bw/d in m/f) consisted of modest reductions in body weight (in lactating F1 females by ca. 10 %) and food consumption, increased spleen weight (by ca. 50 % in both sexes and generations), increased erythropoiesis and haemosiderosis in the spleen, haemosiderosis in the liver and thyroid follicular hyperplasia. The choice of the top dose was based on a preliminary experiment, the results of which are not provided in the main study report.

A slightly reduced litter size at birth was observed in the F1/F2B generation at 250 and 1 250 ppm (see the table below). It is not possible to determine whether the reduction is due to pre- or post-implantation loss from this study, but in another study (B.6.6.1/03) a similar effect was observed in association with a reduced number of implantation sites. The effect is not considered to be of sufficient magnitude to warrant classification. No other effects related to fertility have been identified in this study.

Mean litter size at birth in study B.6.6.1/02				
Dose (ppm)	**0**	**50**	**250**	**1 250**
F0/F1A (± SD)	11.1 (± 2.5)	11.6 (± 1.8)	11.9 (± 1.8)	11.1 (± 1.9)
F0/F1B	11.1 (± 2.6)	11.2 (± 2.0)	10.8 (± 2.6)	11.1 (± 1.9)
F1/F2A	11.2 (± 2.4)	12.3 (± 1.5)	11.0 (± 2.2)	10.8* (± 1.8)
F1/F2B	11.8 (± 2.2)	11.3 (± 2.6)	10.3* (± 1.7)	10.5* (± 2.8)

* according to the study report, 'borderline' statistical significance in the Kruskall-Wallis test

2-generation study B.6.6.1/03

This GLP study was conducted in 1991 generally in line with OECD TG 416 (1983) with several deviations (e.g., lack of histopathological investigations).

Parental toxicity at the top dose of 1 200 ppm (approx. 100/140 mg/kg bw/d m/f) was limited to reductions in body weight (by up to 9 % and 14 % in the P and F1 females respectively) and food consumption. According to the study report, previous studies had indicated that higher concentrations of desmedipham would result in palatability problems.

There was a minor, but statistically significant, reduction in the absolute weight of seminal vesicles in the top dose F0 males (by 11 %). Due to the low magnitude of the effect in F0 generation and absence of the effect in F1 generation and in other studies, this is considered to be an isolated finding not sufficient for classification.

The mean litter size in the F1/F2 generation was slightly reduced due to a reduced number of implantation sites (see the table below). The effect is not considered to be of sufficient magnitude to warrant classification. No other effects related to fertility were identified in this study.

Mean litter size and number of implantation sites in study B.6.6.1/03				
Dose (ppm)	**0**	**100**	**400**	**1 200**
F0/F1				
Mean number of implantation sites; ± SD	15.7 (± 2.1)	16.6 (± 1.9)	16.7 (± 2.4)	16.1 (± 1.9)
Mean litter size	14.1 (± 2.3)	15.8 (± 2.0)	15.3 (± 2.2)	15.0 (± 2.0)
F1/F2				
Mean number of implantation sites	17.0 (± 2.2)	16.7 (± 2.0)	16.4 (± 2.3)	15.7 (± 2.5)
Mean litter size	15.4 (± 2.5)	15.3 (± 1.7)	15.3 (± 2.6)	14.3 (± 2.5)

Conclusion on the classification for fertility and sexual function

Several effects potentially related to fertility and sexual function have been identified in the available multigenerational studies: reduced epididymal sperm count (B.6.6.1/01), delayed puberty onset (B.6.6.1/01) and reduced litter size (B.6.6.1/02, /03). The reductions in litter size are not considered sufficient for classification due to the small size of the effect. The observed reduction in epididymal sperm count and the delayed puberty onset might represent a borderline case for classification. Still, given the magnitude of the effects and the other factors reducing the concern (as discussed above), RAC agrees with the DS that **no classification for adverse effects on sexual function and fertility** is justified.

Adverse effects on development

The available PNDT studies with desmedipham are summarised in the following table.

PNDT studies		
Type of study; Reference; Year	**Method**	**Observations**
Rat		
PNDT study, gavage B.6.6.2/01 2001	OECD TG 414 GLP Strain: Wistar	Maternal toxicity ≤ 500 mg/kg bw/d: no adverse effects Developmental toxicity

	Doses: 0, 10, 100, 500 mg/kg bw/d Dosing GD 6-15 24 females/group	500 mg/kg bw/d: • Delayed ossification (skull, sternebrae) • Liver infarct 100 mg/kg bw/d: • Delayed ossification (sternebrae) • Liver infarct 10 mg/kg bw/d: no adverse effects
PNDT study, gavage B.6.6.2/02 1985	US EPA 83-3 GLP Strain: Wistar Doses: 0, 10, 100, 1 000 mg/kg bw/d Dosing GD 6-15 25 females/group	<u>Maternal toxicity</u> 1 000 mg/kg bw/d: • ↓ food consumption (GD 6-16 by 17 %) and bw gain (GD 6-16 by 51 %); corrected terminal bw reduced by 4 % ≤ 100 mg/kg bw/d: no adverse effects <u>Developmental toxicity</u> 1 000 mg/kg bw/d: • ↓ foetal weight (by 12 %) • Palatoschisis and micrognathia (7 foetuses from the same litter) • Split sternebrae (13 foetuses vs 5 in control) • 1 runt • Reduced ossification • Supernumerary rib (incidence increased 2-fold) 100 mg/kg bw/d: • 1 foetus with agnathia and open eyes, 1 foetus with omphalocele 10 mg/kg bw/d: no adverse effects
PNDT study, gavage B.6.6.2/03 1985 (Follow-up of study B.6.6.2/02)	US EPA 83-3 GLP Strain: Wistar Doses: 0, 10, 100, 500 mg/kg bw/d Dosing GD 6-15 35 females/group	<u>Maternal toxicity</u> 500 mg/kg bw/d: • ↓ food consumption (GD 6-16 by 21 %) and bw gain (GD 6-16 by 43 %); corrected terminal bw reduced by 5 % • Heinz bodies (37 % vs 0 % in controls), ↑ MetHb (9.3 % vs 1.3 % in controls) 100 mg/kg bw/d: • ↑ MetHb (3.7 % vs 1.3 % in controls) 10 mg/kg bw/d: no adverse effects <u>Developmental toxicity</u> 500 mg/kg bw/d: • ↓ foetal weight (by 11 %) • 1 foetus with micrognathia • Reduced ossification 100 mg/kg bw/d: • 1 runt, 1 foetus with hydrops 10 mg/kg bw/d: no adverse effects
PNDT study, gavage	OECD TG 414 GLP	<u>Maternal toxicity</u> 1 000 mg/kg bw/d:

B.6.6.2/05 1991	Strain: Sprague-Dawley Doses: 0, 60, 250, 1 000 mg/kg bw/d Dosing GD 6-16 25 females/group	• ↓ bw gain (GD 6-17 by 29 %) and food consumption (GD 6-17 by 8 %); corrected terminal bw reduced by 3 % • ↑ spleen weight (by 80 %) • Discoloured (brown or purple/black stained) urine in most animals 250 mg/kg bw/d: • ↑ spleen weight (by 19 %); discoloured urine (3 animals) 60 mg/kg bw/d: no adverse effects <u>Developmental toxicity</u> 1 000 mg/kg bw/d: • ↓ foetal weight (by 20 %) • Interventricular septal defect (3 foetuses in 2 litters); partial duplication of inferior vena cava (2 foetuses in 1 litter) • Cleft palate (3 foetuses in 1 litter) • Testis(-es) not fully descended (4 foetuses in 2 litters) • Retarded ossification ≤ 250 mg/kg bw/d: no adverse effects
Rabbit		
PNDT study, gavage B.6.6.2/06 1984	OECD TG 414 GLP Strain: Chinchilla hybrid Doses: 0, 50, 150, 450 mg/kg bw/d Dosing GD 6-27 16 females/group	<u>Maternal toxicity</u> 450 mg/kg bw/d: • ↓ food consumption (GD 6-28 by 26 %); corrected bw reduced by 5 % • 2 abortions (1 total, 1 partial) GD 27-28 150 mg/kg bw/d: • 1 total abortion GD 27 50 mg/kg bw/d: no adverse effects <u>Developmental toxicity</u> 450 mg/kg bw/d: • ↓ foetal bw (by 31 %) • Reduced ossification (phalanges) 150 mg/kg bw/d: • ↓ foetal bw (by 13 %; borderline stat. sign.) 50 mg/kg bw/d: • ↓ foetal bw (by 13 %; borderline stat. sign.)
PNDT study, gavage B.6.6.2/08 1991	OECD TG 414 GLP Strain: New Zealand white Doses: 0, 30, 90, 270 mg/kg bw/d Dosing GD 6-18 16 females/group	<u>Maternal toxicity</u> 270 mg/kg bw/d: • ↓ food consumption (by 49 %) and bw gain (by 29 %) during the dosing period • ↑ spleen weight (by 17 %) • Reduced faecal excretion 90 mg/kg bw/d: • ↑ spleen weight (by 19 %)

		30 mg/kg bw/d: no adverse effects Developmental toxicity 270 mg/kg bw/d: • ↑ early embryonic deaths • ↓ foetal bw (by 7 %) • Retarded ossification • Caudal pelvic shift (13 % vs 1.2 %) 90 mg/kg bw/d: • ↑ early embryonic deaths • Caudal pelvic shift (9.8 % vs 1.2 %) 30 mg/kg bw/d: no adverse effects

Rat PNDT study B.6.6.2/01

This study has been conducted by the same facility as the 2-generation study B.6.6.1/01. The GLP audit on study B.6.6.2/01 found raw data on skeletal observations for a non-existent pup. A study with another substance conducted by this laboratory had previously been considered unreliable by RAC. Similarly to the 2-generation study, although RAC considers study B.6.6.2/01 acceptable for inclusion in the assessment, some doubts about proficiency of the laboratory and accuracy of reporting remain.

No maternal toxicity was observed at the top dose of 500 mg/kg bw/d, although some haematotoxicity can be assumed at this dose based on the results of study B.6.6.2/03. The choice of the top dose was based on results of a range-finding study where 1 000 mg/kg bw/d reportedly caused lethargy, reduced food consumption and macroscopic findings (1-2 out of 5 animals with lung and kidney congestion and mottled liver). The data from the range-finding study are not presented in the main study report.

Developmental findings in the main study are summarised in the table below. The concern about infarct of the liver is notably reduced by the relatively high incidence in concurrent controls. The observed skeletal anomalies, although seen in the absence of maternal toxicity, are considered to be of low toxicological significance. There was no effect on foetal body weight. Overall, the developmental findings in this study are not considered sufficient for classification.

Developmental findings in study B.6.6.2/01				
Dose (mg/kg bw/d)	**0**	**10**	**100**	**500**
Visceral observations – total no. of foetuses (litters)	111 (22)	104 (20)	116 (22)	120 (22)
Liver: infarct; foetuses (litters)	5 (5)	6 (6)	19* (10)	17* (8)
Skeletal observations – total no. of foetuses (litters)	124 (22)	112 (20)	125 (22)	129 (22)
Interparietal: incomplete ossification; foetuses (litters)	4 (3)	7 (4)	4 (3)	15* (9*)
Sternebra: bipartite ossification; foetuses (litters)	1 (1)	1 (1)	7* (5)	9* (5)

* Statistically significantly different from control (p-level not specified, presumably 0.05)

Rat PNDT study B.6.6.2/02

The top dose of 1 000 mg/kg bw/d caused reduced food consumption (by 17 % during the treatment period). The corrected body weight was only marginally reduced (by 4 %, stat. sign.; stat. analysis conducted by RAC). In addition, haematotoxicity is assumed based on the results of the subsequent study B.6.6.2/03.

The most remarkable developmental finding in this study is occurrence of cleft palate and micrognathia in 7 foetuses from the same litter (containing 9 foetuses in total) at the top dose of 1 000 mg/kg bw/d. The severity of micrognathia was 'distinct' in 1 foetus and 'slight' in the remaining 6. General toxicity in the dam producing this litter was not markedly higher compared to other dams of this group. One foetus with agnathia was also observed at the mid-dose of 100 mg/kg bw/d.

In addition, increased incidence of split sternebrae was observed at the top dose; however, presence of split sternebrae in the control group reduces the concern about this anomaly (13 foetuses in 10 litters at the top dose vs 5 foetuses in 5 litters in the control). Likewise, the observed reduction in foetal body weight (by 12 %) and delayed ossification in the presence of some maternal toxicity are not considered sufficiently adverse to warrant classification.

Rat PNDT study B.6.6.2/03

This study was conducted as a follow-up to study B.6.6.2/02 to further investigate the mandibular malformations. Animals of the same strain and source as in study B.6.6.2/02 were used. A lower top dose was chosen (500 mg/kg bw/d instead of 1 000 mg/kg bw/d) to limit the potential confounding effect of maternal toxicity and the group size was increased (35 instead of 25 females per group). In addition, MetHb and Heinz bodies were determined as indicators of haematotoxicity.

The top dose of 500 mg/kg bw/d caused reduced food consumption (by 21 % during the treatment period). The corrected body weight was reduced by 5 % (stat. sign.). A significant increase in MetHb (to 9 %) and Heinz bodies (to 37 %) was observed at the top dose and a small increase in MetHb (to 4 %) was also present at 100 mg/kg bw/d.

No significant developmental findings were observed apart from reduced foetal body weight (by 11 %), delayed ossification, and 1 foetus with micrognathia at the top dose of 500 mg/kg bw/d. This 1 case of micrognathia might be related to the findings of the initial study. The dam with the affected foetus did not show higher toxicity than other dams of this group.

According to the historical control data provided in the study report (time span not specified), there was 1 foetus with agnathia (mandibula) and 1 with cheilognathopalatoschisis (cleft lip, palate and maxilla) among 6 292 control foetuses. The HCD incidence appears to be exceeded at least for mandibular malformations (micrognathia, agnathia) with 3 litters in two consecutive studies (B.6.6.2/02, /03) containing affected foetuses.

Overall, the micrognathia observed in both studies raises concern about developmental toxicity. It is noted that the litter incidences were relatively low and the effect occurred in presence of some maternal toxicity (reduced food consumption, haematotoxicity).

Rat PNDT study B.6.6.2/05

The main manifestations of maternal toxicity at the top dose of 1 000 mg/kg bw/d were effects related to haemolytic anaemia. Most animals showed discoloured urine (indication of

haemoglobinuria and/or haemosiderinuria) on one or more days of gestation and splenic weights were markedly increased (by 80 %). The effects on maternal body weight were limited (corrected maternal bw reduced by 3 %, not stat. sign.).

Foetal weight at the top dose was reduced by 20 %. The reduction in mean foetal weight did not correlate with maternal corrected body weight gain at the level of individual animal data.

Several anomalies were observed at a low incidence at the top dose that were not present in controls or at lower doses. The most notable ones are interventricular septal defect (3 foetuses in 2 litters) and cleft palate (3 foetuses in 1 litter). One of the foetuses with interventricular septal defect had additionally enlarged right atrium and reduced right ventricle. Two cases of interventricular septal defect occurred in a litter with a high post-implantation loss. The dams with the affected foetuses did not show higher general toxicity compared to other dams of this group.

Published HCD (Lang, 1993; the same strain, several laboratories, time span unknown) reported an average foetal incidence of ca. 1:6 000 for ventricular septal defect and ca. 1:10 000 for cleft palate. This indicates that the malformations are relatively rare.

In addition, 2 foetuses from 1 litter showed partial duplication of inferior vena cava. However, as occurrence of this anomaly was limited to one litter and was not seen in three other rat PNDT studies (albeit in a different strain), it is considered to be of less concern than the other malformations that were observed in multiple litters or studies.

Overall, reduced foetal weights and a low incidence of malformations were observed at the top dose in this study where maternal animals suffered from anaemia. Although the maternal anaemia might have contributed to the developmental toxicity, the relationship between maternal and developmental toxicity has not been unequivocally demonstrated. Therefore, interventricular septal defect and cleft palate have to be considered for classification.

Rabbit PNDT study B.6.6.2/06

The choice of the top dose (450 mg/kg bw/d) was based on a dose-range finding study, the results of which are not presented in the main study report. Maternal animals at the top showed reduced food consumption (by 26 %). Terminal body weight corrected for gravid uterus weight was reduced by 5 % (not stat. sign.). The two abortions that occurred on GD 27-28 in the top dose group are likely to be a manifestation of maternal toxicity.

No increase in malformations was observed in this study. Foetal body weight was markedly reduced at the top dose (by 31 %, not fully explained by the concurrent maternal toxicity) and there was also a slight increase in post-implantation loss without a clear dose-response relationship (see the table below).

Post-implantation loss in study B.6.6.2/06

Dose (mg/kg bw/d)	**0**	**50**	**150**	**450**
Food consumption GD 6-28 (g/animal/day)	180	178	177	134
No. of litters, total abortions excluded	13	15	12	14
No. of total abortions	0	0	1	1
Post-implantation loss (%), total abortions excluded; (± SD)	13 (± 14)	18 (± 19)	6 (± 8)	22 (± 23)

Rabbit PNDT study B.6.6.2/08

The top dose of 270 mg/kg bw/d was chosen based on a dose-range finding study where a dose of 360 mg/kg bw/d caused abortion in 3 out of 7 dams. Five out of 7 dams (including those 3 aborting) in the range-finding study showed markedly reduced food consumption (on average by ca. 60 % GD 7-16) and an associated reduction in body weight gain. In view of this marked maternal toxicity at 360 mg/kg bw/d, the top dose in the main study (270 mg/kg bw/d) is considered sufficiently high. Food consumption at the top dose in the main study was reduced by ca. 50 % (in the dosing period), which still indicates significant maternal toxicity. Corrected body weight was reduced by 7 % (not stat. sign.); however, corrected bw is not a suitable indicator of toxicity in this case due to the long interval between the end of dosing (GD 18) and sacrifice (GD 29).

Increased incidence of early embryonic deaths and slight caudal pelvic shift was observed not only at the maternally toxic top dose, but also at the mid-dose, where maternal toxicity was minimal (see the table below). As the increase in early embryonic deaths at the mid-dose of 90 mg/kg bw/d was not marked (2.6-fold) and slight caudal pelvic shift is not considered a malformation, the concern about the developmental findings from this study is by itself not sufficient to trigger classification. However, the early resorptions can be used as additional support for classification triggered by other effects.

Developmental findings in study B.6.6.2/08				
Dose (mg/kg bw/d)	**0**	**30**	**90**	**270**
Food consumption GD 6-18 (kg/animal)	2.3	2.1	2.0	1.1
No. of litters, total abortions excluded	13	12	14	14
No. of total abortions	0	1	0	0
Post-implantation loss (%), total abortions excluded; (± SD)	9 (± 11)	6 (± 9)	14 (± 17)	20 (± 27)
Early resorptions (%); (± SD)	3 (± 6)	3 (± 6)	8 (± 10)	15 (± 26)
Late resorptions and foetal deaths (%); (± SD)	6 (± 8)	2 (± 6)	6 (± 13)	5 (± 7)
Skeletal observations – total no. of foetuses	83	60	82	78
Slight caudal pelvic shift; foetuses (litters)	1 (1)	1 (1)	8 (5)	10 (5)

Multigenerational studies

In study B.6.6.1/01, reduced pup body weight at birth (by 8 %) was observed at the top dose in the F2 generation but due the low magnitude of the reduction, and the fact that it can be at least partly attributed to reduced maternal weight (by 13 % on lactation day 0) it is not considered to support classification.

The DS also discussed increased motor activity in the first 5-10 minutes in F1 males and

F2 females on PND 20 (see the table below) and considered it to provide additional support for classification. The activity in F1 males lacked a dose-response relationship and the control value was rather low compared to other control groups in this study (no HCD is available). The body weights of F2 pups were reduced by 12 % compared to controls on PND 21, potentially leading to a generalised developmental delay and an associated delay in the transient reduction in locomotor activity normally occurring between days 15 and 20 (cf. Bâ and Seri, 1995). Therefore, RAC does not consider this finding to contribute to classification.

Motor activity on PND 20 in the study B.6.6.1/01								
Dose (ppm)	**0**	**50**	**250**	**1 250**	**0**	**50**	**250**	**1 250**
F1	**Males**				**Females**			
Minutes 0-5	281	457**	402*	442**	386	471	436	498
Minutes 5-10	156	239	196	258*	235	244	239	291
Minutes 10-15	100	113	99	162	163	163	119	194
F2	**Males**				**Females**			
Minutes 0-5	462	498	572	563	464	520	596**	621**
Minutes 5-10	263	280	253	317	236	245	305	378**
Minutes 10-15	153	186	152	209	194	129	147	214

Statistically significant difference from control: *, $p \leq 0.05$; **, $p \leq 0.01$

No findings related to developmental toxicity were reported in studies B.6.6.1/02 and B.6.6.1/03.

Summary of developmental effects

RAC has identified the following findings as potentially relevant for classification in the available studies:

- Micrognathia (B.6.6.2/02, /03)
- Cleft palate (B.6.6.2/02, /05)
- Interventricular septal defect (B.6.6.2/05)
- Early resorptions (B.6.6.2/08)

Micrognathia was observed in two studies conducted in the same strain by the same laboratory. In the first study (B.6.6.2/02) it occurred at 1 000 mg/kg bw/d in 7 foetuses of the same litter. In the follow-up study (B.6.6.2/03), 1 foetus with micrognathia was observed at the top dose of 500 mg/kg bw/d. In addition, 1 foetus with agnathia was found in the initial study at 100 mg/kg bw/d. Mandibular malformations were very rare in the historical control data. Maternal toxicity at the doses with micrognathia consisted of reduced food consumption (by ca. 20 %) and haematotoxicity (MetHb 9 % at 500 mg/kg bw/d, presumably also reduced Hb).

Cleft palate was observed in two studies (B.6.6.2/02 and /05) in two different strains (Wistar and Sprague-Dawley). Cleft palate is a very rare malformation in both strains. RAC notes the publication by Price *et al.* (2016) suggesting that micrognathia causes cleft palate in animals and humans. Thus, the cleft palates in study B.6.6.2/02 may by causally linked to

micrognathia observed in the same foetuses. In each study, the occurrence of cleft palate was limited to 1 litter at 1 000 mg/kg bw/d. Significant maternal haematotoxicity was observed in study B.6.6.2/05 (discoloured urine, markedly increased spleen weight) and methaemoglobinaemia together with reduced Hb are likely to have been present in study B.6.6.2/02.

Interventricular septal defect was observed in one study (B.6.6.2/05) in 3 foetuses from 2 litters at 1 000 mg/kg bw/d. Again, this was a dose associated with maternal haematotoxicity.

Early resorptions, increased 2.6-fold in one of the rabbit studies (B.6.6.2/08) at a dose without significant maternal toxicity, can be used as additional support for classification.

Overall, several malformations were observed at a low incidences in the rat studies at doses with maternal haematotoxicity. It is possible that maternal anaemia might have contributed to a certain extent to some of the observed developmental findings (cf. Webster and Abela, 2007). However, a causal relationship between maternal and developmental toxicity has not been unequivocally demonstrated.

Conclusion on classification for development

Several malformations of high concern (micrognathia, cleft palate, interventricular septal defect) were observed at low incidences in the rat PNDT studies at doses associated with maternal anaemia. Although it cannot be excluded that maternal toxicity has contributed to these effects, unequivocal evidence for a causal relationship between maternal and developmental toxicity is missing.

Occurrence of malformations can in principle lead to classification in Category 1B. However, taking into account the low incidences and concurrent maternal toxicity, RAC agrees with the DS **to classify as Repr. 2; H361d for development**.

Consideration of setting a specific concentration limit (SCL)

As the effects triggering classification were generally observed at high doses, indicating low potency, RAC has discussed setting an SCL. SCLs are derived according to the procedure described in the CLP guidance (section 3.7.2.6). In the first step, a preliminary potency group is assigned based on ED_{10} values or, if not available, LOAELs for the effects triggering classification. In the next step, the final potency group is selected after consideration of modifying factors. The relevant effects together with their ED_{10} or LOAEL values are summarised in the table below (since for none of the malformations an ED_{10} could be reached due to low incidence, LOAELs were used instead).

Effect (species)	**ED_{10} or LOAEL**	**Preliminary potency group**	**Studies where the effect was observed**
Main effects triggering classification			
Cleft palate (rat)	1 000 mg/kg bw/d (LOAEL)	Low	B.6.6.2/02, /05
Interventricular septal defect (rat)	1 000 mg/kg bw/d (LOAEL)	Low	B.6.6.2/05
Micrognathia (rat)	500 mg/kg bw/d (LOAEL)	Low	B.6.6.2/02, /03
Effects providing additional support for classification			
Early resorptions (rabbit)	220 mg/kg bw/d (ED_{10})	Medium	B.6.6.2/08

Agnathia (rat)	100 mg/kg bw/d (LOAEL)	Medium	B.6.6.2/02

The only relevant modifying factor is severity of effect. If a severe effect is observed close to the border of a higher potency group, the higher potency group should be considered. Such a modification is applicable to micrognathia in rat occurring from 500 mg/kg bw/d. Thus, a medium potency group could be considered for this effect. The supporting effects such as agnathia and early resorptions also correspond to the medium potency group. Therefore, RAC decided not to recommend a specific concentration limit for the developmental toxicity of desmedipham.

Adverse effects on or via lactation

Although the DS did not evaluate this endpoint due to lack of data, some information on adverse effects on or via lactation can be obtained from the multigenerational studies.

In study B.6.6.1/01, the F2 pup body weight was reduced by 8 % at birth and by 11 % on PND 7 compared to controls (top dose: 1 250 ppm). The effect can be at least partly explained by maternal toxicity at this dose (maternal weight reduced by 13 % and 11 % on LD 0 and 7 respectively).

In study B.6.6.1/02, body weight of the F2B pups was reduced by 17 % on PND 7 compared to controls (top dose: 1 250 ppm). Maternal body weight was reduced by 11 % at that time. The pup body weight reduction of this magnitude was transient (reduction by 5 %, 6 %, 17 %, 8 % and 8 % on PND 0, 4, 7, 14 and 21 respectively) and was not seen in F2A pups (bw on PND 7 reduced by 8 %).

In study B.6.6.1/03, body weight of the pups on PND 7 was reduced by ca. 11 % and 8 % compared to controls in the F1 and F2 generation respectively (top dose: 1 200 ppm). The pup body weight at birth was not affected. Maternal weights of P and F1 dams on PND 7 were decreased by 5 % and 11 % respectively.

No other effects potentially related to lactation were reported in these studies.

Conclusion on classification for lactation

The reductions in pup body weight attributable to lactation seen in the generational studies with desmedipham are not considered to be of sufficient magnitude to warrant classification, or there are other factors reducing the concern (maternal toxicity, transient nature of the reduction). Therefore, RAC proposes **no classification for effects on or via lactation**.

Overall conclusion on reproductive toxicity

RAC agrees with the DS that desmedipham should be **classified as Repr. 2; H361d**.

10.11 Specific target organ toxicity-single exposure

10.12 Specific target organ toxicity-repeated exposure

Table 25 Summary table of short-term animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results		Reference
		NOAEL	LOAEL	
Rats				
5-week dose range finding study in rat no guideline, no GLP rat, Sprague-dawley 10/sex/group	desmedipham technical (Purity: 99.3%) oral, diet 0, 2000, 4000 ppm M/F: 0, 133, 266 mg/kg bw/day exposure: 5 weeks	< 2000 ppm M: < 194.5 mg/kg bw/day F: < 232.0 mg/kg bw/day	2000 ppm Reduced body weight gain and food consumption	1989 RAR B.6.3.2/01 M-147174-01-1
90-day oral toxicity study in rat EPA guideline 82-1 and comply with OECD Guideline No. 408 GLP rat, Wistar 10/sex/group	desmedipham technical (Purity: 97.8%) oral, diet 0, 300, 1200, 4800 ppm M: 0, 24, 97, 415 mg/kg bw/day F: 0, 27, 109, 378 mg/kg bw/day exposure: 13 weeks	< 300 ppm M: < 24 mg/kg bw F: < 27 mg/kg bw/day	300 ppm in both sexes ↑ Methemoglobinemia, ↑ hematopoiesis in in liver and spleen. No NOAEL	1984a RAR B.6.3.2/02 M-146746-01-1
90-day oral toxicity study in rat EPA guideline 82-1 and comply with OECD Guideline No. 408, GLP rat, Wistar 25/sex/group Microscopical examination of all tissues were performed only in control and high dose animals, although treatment related changes were observed e.g. in spleen and mandibular lymph nodes (increased incidence in high dose or findings in prematurely deceased animals).	desmedipham technical (Purity: 98.3%) oral, diet 0, 6, 30, 60, 300 ppm M: 0, 0.5, 2.6, 5.2, 26 mg/kg bw/day F: 0, 0.5, 2.7, 5.6, 27 mg/kg bw/day exposure: 13 weeks	30 ppm M: 2.6 mg/kg bw /day F: 2.7 mg/kg bw /day	60 ppm ↑ methemoglobin seen at 60 ppm Additional effects at 300 ppm: Slight hematological effects ↑ erythropoiesis in spleen, ↑ in levels of methemoglobin and reticulocytes in both sexes. ↓ T4 levels	1985 RAR B.6.3.2/03 M-146760-01-1

90-day oral toxicity study in rat OECD Guideline No. 408, GLP rat, Sprague Dawley 10/sex/group Mammary glands and seminal vesicles were excluded from the histopathological examination	desmedipham technical (Purity: 99.3%) oral, diet 0, 160, 800, 4000 ppm M: 0, 10.6, 54, 275 mg/kg bw/day F: 0, 12.3, 60, 339 mg/kg bw/day exposure: 13 weeks	< 160 ppm M: <10.6 mg/kg bw/day F: 12.3 mg/kg bw/day	160 ppm ↑ congestion of the spleen, changes in red cell parameters in females and morphological changes in red cell in both sexes at 800 ppm.↑ enlargement and hemosiderosis was observed in spleen and minimal to moderate follicular cell hypertrophy in thyroids in both sexes at 800 ppm.	1987 RAR B.6.3.2/04 M-146976-01-1
Mice				
28-day oral toxicity study in mouse US EPA guideline 82-1 comply with OECD guideline 407, GLP mouse, NMRI 10/sex/group	desmedipham technical (Purity: 97.8%) oral, diet 0, 100, 400, 1600 ppm M: 0, 22, 91, 416 mg/kg bw/day F: 0, 26, 108, 519 mg/kg bw/day exposure: 28 days	100 ppm M: 22 mg/kg bw/day F: 26 mg/kgbw/day	400 ppm Methemoglobinemia/Hemolytic anemia (Heinz bodies↑ Met-Hb↑) Hematopoiesis in spleen	1984b RAR B.6.3.1/01 M-146747-01-1
90-day dose range finding study in mouse no guideline, GLP mouse, Crl:CD-1 10/sex/group	desmedipham technical (Purity: 97.5%) oral, diet 0, 750, 1300, 2300, 4000 ppm M: 0, 134, 209, 402, 714 mg/kg bw/day F: 0, 148, 237, 484, 1008 mg/kg bw/day exposure: 13 weeks	750 ppm M: 134 mg/kg bw /day F: 148 mg/kg bw/day	1300 ppm Hemolytic anemia (RBC↓ Hb ↓ Hct ↓ MCHC↑ MCH↑, reticulocytes↑) Increased organ weights (spleen, liver, kidneys)	1992 RAR B.6.3.2/05 M-146977-01-1
Dogs				
28-day dose range finding study in dog no guideline, GLP Beagle dog 1/sex/group	Desmedipham (Purity: 97.8%) oral, diet 0, 200, 1000, 5000/ 7500 ppm M/F: 0, 7, 30, 151/214 mg/kg bw/day exposure: 28 days	200 ppm 7 mg/kg bw /day	1000 ppm Hemolytic anemia (↓ erythrocytes and hemoglobin, ↑ in reticulocytes, MCHC, MCV, MCH,)	1984 RAR B.6.3.2/06 M-146733-01-1

42-day oral toxicity study in dog no guideline, GLP Beagle dog three males/group no histopathology	Desmedipham (Purity: 98.4%) oral diet 0, 1, 15 ppm M: 0, 0.036, 0.483 mg/kg bw/day exposure: 42 days	> 0.483 mg/kg bw/day Inconclusive hematological effects	Inconclusive hematological effects	1985 RAR B.6.3.2/07 M-146761-01-1
90-day oral toxicity study in dog OECD Guideline 409, GLP Beagle dog 4/sex/group epididymides, uterus and thymus in all animals were not weighed. Stability of desmedipham in the food was investigated in another study	desmedipham technical (Purity: 98.3%,) oral, diet 0, 1, 5, 150 ppm M: 0.035, 0.17 or 4.97, mg/kg/day F: 0.035, 0.19 or 5.50 mg/kg/day for both M/F: 0, 0.035, 0.18, 5.24 mg/kg bw/day exposure: 90 days	>150 ppm M/F > 5.24 mg/kg bw/day	>5.24 only transitional increases in methemoglobin levels at 150 ppm but with no decreases in RBC counts, Heinz bodies or hemoglobin levels	1986 RAR B.6.3.2/08 M-146762-01-1
90-day oral toxicity study in dog OECD Guideline 409, GLP Beagle dog 4/sex/group Histopathology was not always adequate. For example, hypercellularity in bone marrow was observed in females at 1500 ppm, but this tissue was not systematically examined from lower dose groups. Epididymides were not weighed and assessory sex organs were not examined histopathologically.	desmedipham technical (Purity: 98%) oral, diet 0, 100, 500, 1500 ppm M: 0, 3.7, 18.7, 56.7 mg/kg bw/day F: 0, 4.1, 21.1, 62.3 mg/kg bw/day exposure: 13 weeks	100 ppm M: ca. 18.7 mg/kg bw/day F: 4.1 mg/kg bw/day	1500 ppm (M) and 500 ppm (F) ↑ Minimal/mild follicular epithelial hypertrophy in thyroids and increased thyroid weight	1991 RAR B.6.3.2/09 M-146978-01-1

1-year oral toxicity study in dog OECD Guideline 452, GLP Beagle dog 6/sex/group unclear whether all tissues were examined and findings reported	desmedipham technical (Purity: 97.8%) oral, diet 0, 300, 1500 7500/5000 ppm M: 0, 9.6, 52.5, 167.7 mg/kg bw/day F: 0, 10.4, 57.4, 200.7 mg/kg bw/day exposure: 12-months	300 ppm M: 9.7 mg/kg bw/day F: 10.4 mg/kg bw/day	1500 ppm marked iron deposits in the liver primarily at 1500 ppm and higher doses, ↑ erythropoiesis in the bone marrow at 1500 ppm	1985 RAR B.6.3.2/10 M-146756-01-1
80-day oral toxicity study in dog no guideline, GLP The aim to establish no-effect level for methemoglobin formation in dog Beagle dog 2/sex/group	desmedipham technical (Purity: 97.6%) oral, diet Increasing or varying dosage at 150/200/500 or 75/300/0/1500 ppm, about 20-40 days/dose level, over a total of 80 days	"NOAEL" M/F: 300 ppm = 9.7-11.1 mg/kg bw/day	No true NOAEL can be derived, but the study indicates that Met-Hb is only moderately increased using this type of exposure. No effects on other RBC parameters observed	1991 RAR B.6.3.2/11 M-146807-01-1
28-day oral toxicity study in the rat no guideline, no GLP to evaluate the mode of action for thyroid findings rat, Wistar 15 male/group	Desmedipham technical (Purity: 95.5 %) oral, diet 1500 and 4000 ppm 94 and 252 mg/kg bw/day exposure: 28 days	-	Liver enzyme induction. Desmedipham treatment up-regulates Ugt2b1 transcript from the UDP glucuronosyltransferase gene family in rats.	2014 RAR B.6.8.2/01 M-489615-01-1

M= male, F= female, RBC = red blood cell, Hct = haematocrit, Hb = hemoglobin, MetHb = methemoglobin, MCHC = mean corpuscular heamoglobin concentration, MCV = mean corpuscular volume and MCH = mean corpuscular haemoglobin

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Thirteen short-term toxicity studies are available on desmedipham (Table 25). Chronic toxicity/carcinogenicity studies and reproductive toxicity studies are reported in the sections 10.9 and 10.10. Further details are given in RAR.

Short-term repeated dose toxicity of desmedipham has been investigated in mice, rats and dogs using oral administration. Generally, the studies were old (from the 80'ties and 90'ties) and therefore not fully in compliance with the current OECD test guidelines. This means that some histopathological examinations which are currently standard are missing. New endpoints e.g. for neurotoxicity (auditory, visual and proprioceptive stimuli), assessment of grip strength and motor activity assessment should be conducted in short-term studies but were not assessed in the studies.

<u>Rats</u>

Subchronic effects in rats were examined in four 90-day studies. Hematological effects, mainly changes in red blood cell parameters were observed in all rat studies. The main hematological effects consisted of increased methemoglobin levels, reticulocytes, formation Heinz bodies, mean cellular hemoglobin concentrations, mean cell volumes and mean cell hemoglobin levels, and decreased red blood cell counts, hemoglobin concentrations and hematocrit. Morphological changes in red blood cells were also noted. The observed effects in red blood cell parameters were indicative of hemolytic anemia.

The observed increases of methemoglobin levels (% of Hb) ranged from 1.4 % at 27 mg/kg bw (300 ppm) in females vs 0.8 % in controls (1985, RAR B.6.3.2/03) up to 11.2 % at 415 mg/kg bw in males (4800 ppm) vs 1.0 % in controls (1984a, RAR B.6.3.2/02), respectively. The highest reduction in hemoglobin (Hb) level in blood was up to 15 % in comparison with controls at 339 mg/kg bw/day (4000 ppm) in the study RAR B.6.3.2/04 (1987).

The study RAR B.6.3.2/03 (1985) showed that the observed methemoglobinemia was partly reversible during four weeks of recovery period, since in males only slight methemoglobinemia at the two highest doses (60 ppm and 300 ppm) of 1.0 and 1.1 % vs 0.8 % in the control remained, whereas in females it was fully reversible. However, since the dose levels were low and effects on hematology only slight to begin with, it was difficult to draw conclusions on the actual reversibility of the condition induced by treatment with desmedipham.

Organ weight changes usually involved increased liver, spleen and/or kidney weights. Increased thyroidal weights were also observed, as were decreases in T4 levels.

Histopathological findings in rats were mainly related to the observed hematological changes and were consistent with compensatory erythrogenic responses. Extramedullary hematopoiesis was observed in spleen and liver. Deposition of brown pigment, sometimes identified as iron-positive (hemosiderin), was noted in liver, spleen and kidneys. Congestion of the spleen was noted in males at 10.6 mg/kg bw/day (160 ppm), 54 mg/kg bw/day (800 ppm) and at 275 mg/kg bw/day (4000 ppm) in the study RAR B.6.3.2/04 (1987). Congestion of the spleen was also noted in females at 60 mg/kg bw/day (800 ppm) and at 339 mg/kg bw/day (4000 ppm). In the study RAR B.6.3.2/02 (1984a) in most males at dose level of 415 mg/kg bw (4800 ppm) the spleen was enlarged and dark red to black in color. The overall NOAEL in rat was 2.6 mg/kg bw/day, based on changes in hematology consistent with hemolytic anemia.

Thyroidal follicular cell hyperplasia or hypertrophy was observed in studies RAR B.6.3.2/02 (1984a) and RAR B.6.3.2/04 (1987).

Induction of liver enzymes in rats after oral treatment with desmedipham were examined in a 28-day toxicity study in the rat by dietary administration (2014, RAR B.6.8.2/01). Desmedipham treatment up-regulated Ugt2b1 transcript from the UDP glucuronosyltransferase gene family in rats indicating liver enzyme induction.

Some decreases in plasma and brain cholinesterase were noted in two rat subchronic studies (1984a, RAR B.6.3.2/02;1987, RAR B.6.3.2/04). However, given the small magnitude of the difference in brain cholinesterase and the considerable variability of the results in the assays, these differences were not considered to be toxicologically significant.

Mice

One 28-day study and one 90-day study in mouse was performed, both by oral administration. Again, hematological effects consistent with hemolytic anemia were seen. Hemoglobin (Hb) level in blood was reduced up to 10 % in males in comparison with controls at 416 mg/kg bw/day in the 28-day study (1984b, RAR B.6.3.1/01). Formation of Heinz bodies and methemoglobin (% of Hb) were significantly increased at 91 – 108 mg/kg bw/day (in males 5.1 vs 1.8 % and in females 3.5 vs 1.3 %, respectively). The highest percentage of methemoglobin in blood in males was 14.3% at 416 mg/kg bw/day vs 1.8 % in controls. High dose females had methemoglobin levels of 6.0 % at 519 mg/kg bw vs 1.3 % in controls and a slightly increased level (3.5 %) at the next lower dose of 108 mg/kg bw. Reticulocytes were increased at the highest tested dose. Extramedullar hematopoiesis in the spleen of both sexes and increased erythropoiesis (increased red cell precursors) in the bone marrow of males was observed.

Changes in red blood cell parameters were noted especially in females at 237 mg/kg bw/day and higher doses in the 90-day study (1992, RAR B.6.3.2/05). Increased spleen weights were observed especially in females at 237 mg/kg bw/day and higher doses. Kidney and liver weights were also increased in females at 484 and 1008 mg/kg bw/day. The overall NOAEL for effects in mice was 22 – 26 mg/kg bw/day, based on changes in red blood cell parameters (hemolytic anemia) and related effects.

Dogs

Subchronic effects in dogs were examined in six dog studies. Hematological effects consistent with hemolytic anemia were observed also in dogs.

Changes in red blood cell parameters consistent with hemolytic anemia were observed at 52.5-57.4 mg/kg bw/day (1500 ppm) and higher doses in a one-year dog study (1985, RAR 6.3.2/03). Hemoglobin (Hb) level in blood was reduced 40 % in females in comparison with controls at 200.7 mg/kg bw/day (7500/5000 ppm) after 13 weeks and 23 % after 52 weeks. In males Hb was reduced 24 % after 13 weeks and 20 % after 52 weeks at 7500/5000 ppm. The highest level of methemoglobin (% of Hb) in males was 8.5% vs 0.8 % in controls at 167.7 mg/kg bw/day (7500/5000 ppm) after 52 weeks. Three dogs (2 males, 1 female) in 5000 ppm dose groups died or had to be killed during the course of the study. The main pathology findings in these dogs were anemia and lesions secondary to anemia (erythropoiesis in spleen, increased erythropoiesis in the bone marrow, bone marrow atrophy, and iron deposition in Kupffer cells of the liver). Increased liver and kidney weights were noted at 7500/5000 ppm, and thyroid weights were increased in females at 1500 and 7500/5000 ppm. Extramedullar hematopoiesis in the spleen was increased at 7500/5000 ppm, and increased erythropoiesis was noted in both sexes at doses between 1500 and 7500/5000 ppm. Congestion (slight and moderate) of the spleen was noted in males (2/4) at 5000/7500 ppm. Increased follicular hyperplasia of the thyroid was observed at 7500/5000 ppm in males and in 1500 and 7500/5000 ppm females. NOAEL was 1500 ppm based on iron deposits in the liver and increased bone marrow erythropoiesis at 1500 ppm and higher doses.

Slightly but significantly increased levels of methemoglobin begin to appear consistently in dogs below a dose level of 10 mg/kg bw/day (1991, RAR B.6.3.2/11; 1984, RAR 6.3.2/06) but was transiently observed at lower doses too down to a dose of 5.42 mg/kg bw/day (1986, B.6.3.2/08). Only transient hematological effects (red blood cell parameters) were observed in the study RAR B.6.3.2/09 (1991) with doses up to 1500 ppm (approximately 56.7 – 62.3 mg/kg bw/day). A study RAR B.6.3.2/11 (1991) with increasing/varying levels of desmedipham showed that levels of methemoglobin were only slightly increased (maximum of 1.4%), and varied extensively, at doses up to 1500 ppm during periods of time in excess of 20 days per dose level. Extramedullar hematopoiesis in the spleen and hypercellularity in bone marrow was slightly increased in 1500 ppm females in the study RAR B.6.3.2/09.

Pituitary cysts were noted in female dogs in a study B.6.3.2/08 (1986). This histopathological finding was not confirmed by other studies in dog. In the study RAR B.6.3.2/09 (1991) follicular cell hyperplasia in thyroids was noted in females at doses of 500 and 1500 ppm and in males at 1500 ppm (RAR B.6.3.2/09). Thyroid glands were also increased in weight at 1500 ppm. The overall NOAEL in dog was 4.1 mg/kg bw/day, based on increased follicular cell hyperplasia in thyroids in dogs.

UDP-glucuronosyltransferase (UGT) induction in liver, Cytochrome P450 (CYP) and UGT mRNA expression levels were measured in an in vitro liver model, i.e. DMP-treated dog hepatocytes (2017, RAR B.6.8.2/02). Desmedipham treatment up-regulated CYP2B6 and CYP3A4, and also UGT2B31 mRNA in dog hepatocytes like phenobarbital.

10.12.2 Comparison with the CLP criteria

As described above, target organs of toxicity in experimental animals following repeated oral administration of desmedipham are blood and thyroid gland. In addition, effects on acetylcholinesterase activity was

observed. However, the relevancy of these findings has to be weighted and compared with the CLP classification criteria.

According to CLP regulation (EC) No 1272/2008, substances are classified for target organ toxicity STOT RE 1 if they have produced significant toxicity in humans or, on the basis of evidence from studies in experimental animals, they can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.

Substances are classified in Category 2 for target organ (STOT RE 2) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. On the basis of evidence from studies in experimental animals it can be presumed that the substance has the potential to be harmful to human health following repeated exposure.

For classification based on the results obtained from studies conducted in experimental animals guidance values are given to discriminate low and moderate exposure doses/concentrations. Guidance values, are not, however, intended as strict demarcation values but are given only for guidance purposes, i.e., to be used as part of the weight of evidence approach, and to assist with decisions about classification. Guidance values are given for 90 days studies and can be adjusted for studies with shorter or longer duration by using the Haber's rule.

Effects considered to support classification for specific target organ toxicity following repeated exposure are given in point 3.9.2.7 of the CLP Regulation.

Haematological effects

In order to be classified according to CLP a substance should cause any consistent and significant adverse changes in biochemistry, haematology, or urinalysis parameters (Section 3.9.2.7.3. c). The Guidance on the Application of the CLP criteria (ECHA, 2017) gives some additional guidance on the evaluation of haemolytic anemia. A classification is warranted, if a haemolytic substance induces one or more of the serious health effects listed below as examples within the critical range of guidance values given. It is sufficient for classification that only one of these criteria is fulfilled.

- Premature deaths in anaemic animals that are not limited to the first three days of treatment in the repeated dose study (Mortality during days 0–3 may be relevant for acute toxicity).
- Clinical signs of hypoxia, e.g. cyanosis, dyspnoea, pallor, in anaemic animals that are not limited to the first three days of treatment in the repeated dose study.
- Reduction in Hb at ≥20%.
- Reduction in functional Hb at ≥20% due to a combination of Hb reduction and MetHb increase.
- Haemoglobinuria that is not limited to the first three days of treatment in the repeated dose study in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥10%).
- Haemosiderinuria supported by relevant histopathological findings in the kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥10%).
- Multifocal or diffuse fibrosis in the spleen, liver or kidney.
- Tubular nephrosis
- Marked increase of haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥10%) in a 28 day study.
- Significant increase in haemosiderosis in the spleen, liver or kidney in combination with microscopic effects like necrosis, fibrosis or cirrhosis.

The guidance on the application of the CLP criteria also states that in the case where multiple less severe effects with regenerative capacity were observed, the classification should apply as "Assessment shall take

into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs." (CLP Annex I, 3.9.1.4).

It should be noted that as defined in point 3.9.2.8.1. of CLP there are some insignificant hematological effects in humans and/or animals that do not justify classification. Such effects include, but are not limited to:

- small changes in clinical biochemistry, haematology or urinalysis parameters and/or transient effects, when such changes or effects are of doubtful or minimal toxicological importance
- Significant decrease in Hb without any other significant indicators of haemolytic anaemia.
- Minimal to slight increase in MetHb formation without any other indications of significant haemolytic anaemia.
- Only adaptive or compensating effects without significant signs of haemolytic anaemia.

The following tables (Table 26 and Table 27) and summarises the key haematological findings from relevant studies as they relate to the guidance values for STOT-RE classification. The information on effects after short-term repeated exposure was complemented by the non-neoplastic results from the combined chronic toxicity and carcinogenicity studies in mice and rats. In addition, the maternal toxicity data of the oral developmental toxicity studies in rats and rabbits and of a two-generation toxicity studies in rats were considered for the evaluation of the specific target organ toxicity after repeated exposure of desmedipham.

Table 26 Summary table of haematological findings in relevant short-term repeated dose toxicity studies

Study	**Doses relevant for STOT RE classification**	**Effects at this dose level**
Rats		
90-day oral toxicity study in rat EPA guideline 82-1 and comply with OECD Guideline 408, GLP rat, Wistar 10/sex/group, oral (diet) 0, 300, 1200, 4800 ppm M: 0, 24, 97, 415 mg/kg bw/day F: 0, 27, 109, 378 mg/kg bw/day Blood samples for haematological measurements were collected after 13 weeks of treatment 1984a RAR B.6.3.2/02 M-146746-01-1	*Cat 2: 10 < C ≤ 100 mg/kg bw/day*	at 300 ppm - ↓ Hb (5 %* in males and 4 %* in females), RBC (5 %* in males and 7%** in females) and Hct (4 %** in males and 4 %* in females) - ↑ MetHb 170 %** in males and 91 %** in females compared to controls (% of Hb: 2.7** vs 1.0 in controls and 2.1** vs 1.1 in controls) - ↑ reticulocytes (50 %**) in females - ↑ haematopoiesis (mainly erythropoiesis) in the spleen (minimal to slight) in males and females (9/10 and 9/10 vs no incidence in controls) - morphological changes in erythrocytes as polychromatophilia in males at 1200 ppm - ↓ Hb (9 %** in males and 11%** in females), RBC (12%** in males and 17%** in females) and Hct (9 %** in males and 4** in females - ↑ MetHb 430 %** in males and 264 %** in females compared to controls (% of Hb: 5.3** vs 1.0 in controls and 4.0** vs 1.1 in controls) - ↑ reticulocytes (126 - 186 %**) in males and females - ↑ Heinz bodies (‰) in males and females (33* and **7** vs no incidence in controls)

		- morphological changes in the erythrocytes as polychromatophilia and anisocytosis in both sexes - ↑ extramedullary haematopoiesis in the liver (slight) in males (2/10 vs no incidence in controls) - ↑ haematopoiesis (mainly erythropoiesis) in the spleen (minimal to moderate) in males and females (10/10 and 10/10 vs no incidence in controls) - iron-positive pigmentation of Kupffer cells (minimal to slight) in males and females (4/10 and 1/10 vs no incidence in controls) - iron-positive pigmentation in kidneys (minimal) in males (2/10 vs 0/10 in controls) - ↑ kidney and liver weight in females (relative 18 %** and 12 %**)
90-day oral toxicity study in rat EPA guideline 82-1 and comply with OECD Guideline 408, GLP rat, Wistar 25/sex/group, oral (diet) 0, 6, 30,60, 300 ppm M: 0, 0.5, 2.6, 5.2, 26 mg/kg bw/day F: 0, 0.5, 2.7, 5.6, 27 mg/kg bw/day Blood samples for haematological measurements were collected at 4/5, 9 and 12/13 weeks of treatment and from the recovery group at 16/17 weeks 1985 RAR B.6.3.2/03 M-146760-01-1	*Cat 1: C ≤ 10 mg/kg bw/day* *Cat 2: 10 < C ≤ 100 mg/kg bw/day*	at 60 ppm - ↑ MetHb up to 50 %* in males and up to 38 %* females compared to controls (% of Hb: 1.2* vs 0.8 in controls and 1.1* vs 0.8 in controls) and also lasting in the recovery period in males - ↓ liver weight in females (absolute and relative 11 %**) at 300 ppm - ↓ Hb (up to 6 %* in males) . Also, lasting in the recovery period in males - ↑ MetHb up to 338 %* in males and 238 %* in females compared to controls (% of Hb: 3.5* vs 0.8 and 2.7* vs 0.8 in controls) and also lasting in the recovery period in males - ↑ reticulocytes in males and females (statistically significant) - ↑ erythropoiesis in the spleen (minimal to slight) in males (14/15 vs 3/15 in controls). No change in females (13/15 vs 12/15 in controls).
90-day oral toxicity study in rat OECD Guideline 408, GLP rat, Sprague Dawley 10/sex/group, oral (diet) 0, 160, 800, 4000 ppm M: 0, 10.6, 54, 275 mg/kg bw/day F: 0, 12.3, 60, 339 mg/kg bw/day Blood samples for haematological measurements were collected during week 13 1987	*Cat 2: 10 < C ≤ 100 mg/kg bw/day*	at 160 ppm - congestion of the spleen in males (6/10 vs 0/10 in controls) at 800 ppm - ↓ Hb in females (3 %*) and in males (4 %) - ↓ RBC (8 %**) in females - congestion of the spleen in males and females (9/10 and 3/10 vs no incidence in controls). Severity was not reported. - ↑ hemosiderosis (minimal) in the spleen in males and females (4/10 vs 0/10 in controls and 9/10 vs 3/10 in controls)

RAR B.6.3.2/04 M-146976-01-1		
Mice		
28-day oral toxicity study in mouse US EPA guideline 82-1 and comply with OECD guideline 407, GLP mouse, NMRI 10/sex/group, oral (diet) 0, 100, 400, 1600 ppm M: 0, 22, 91, 416 mg/kg bw/day F: 0, 26, 108, 519 mg/kg bw/day Blood samples for haematological measurements were collected after 4 weeks of treatment 1984b RAR B.6.3.1/01 M-146747-01-1	*Cat 1: C ≤ 30 mg/kg bw/day* *Cat 2: 30 < C ≤ 300 mg/kg bw/day*	at 400 ppm - ↓ Hb (6 %*) in males - ↑ MetHb 170 %** in females and 183 % in males compared to controls (% of Hb: 3.5** vs 1.3 and 5.1 vs 1.8 in controls) - ↑ Heinz bodies (o/oo) in males and females (368** vs 28 in controls and (247** vs 21 in controls) - haematopoiesis (mainly erythropoiesis) in the spleen (minimal to slight) in males and females (3/10 and 2/10 vs no incidence in controls) - morphological changes in erythrocytes (increased anisocytosis in the males at 100 ppm and in both sexes at 400 ppm)
Dogs		
28-day dose range finding study in dog no guideline, GLP Beagle dog 1/sex/group, oral (diet) 0, 200, 1000, 5000/ 7500 ppm M/F: 0, 7, 30, 151/214 mg/kg bw/day Blood samples for haematological measurements were collected for pretest and after 4 weeks of treatment 1984 RAR B.6.3.2/06 M-146733-01-1	*Cat 1: C ≤ 30 mg/kg bw/day* *Cat 2: 30 < C ≤ 300 mg/kg bw/day*	at 1000 ppm - ↓ Hb and RBC - ↑ reticulocytes, MCHC and MCV, MCH at 5000/7500 ppm - ↓ Hb and RBC - ↑ reticulocytes, MCHC, MCV and MCH - trends for higher liver weight in the male as well as higher spleen weight in the female (5000/7500 ppm).
42-day oral toxicity study in dog no guideline, GLP Beagle dog, three males/group oral (diet) 0, 1, 15 ppm M: 0, 0.036, 0.483 mg/kg bw/day Blood samples for haematological measurements were collected weekly during the study 1985 RAR B.6.3.2/07	*Cat 1: C ≤ 20 mg/kg bw/day*	at 1-15 ppm - inconclusive haematological effects - ↑ spleen weight at 1 ppm (absolute 17 %)

M-146761-01-1		
90-day oral toxicity study in dog OECD Guideline 409, GLP Beagle dog, 4/sex/group oral (diet) 0, 1, 5, 150 ppm M: 0.035, 0.17 or 4.97, mg/kg/day F: 0.035, 0.19 or 5.50 mg/kg/day for both M/F: 0, 0.035, 0.18, 5.24 mg/kg bw/day Blood samples for haematological measurements were collected before the treatment and days 15, 29, 43, 57, 71 and 85 of treatment 1986 RAR B.6.3.2/08 M-146762-01-1	*Cat 1: C ≤ 10 mg/kg bw/day*	at 150 ppm - transient ↑ of MetHb up to 89 %* in males and 50 %* in females compared to controls (% of Hb: up to 1.7* vs 0.9 and 1.5* vs 1.0 in controls) - no effects on RBC counts, Heinz bodies or Hb levels
90-day oral toxicity study in dog OECD Guideline 409, GLP Beagle dog, 4/sex/group oral (diet) 0, 100, 500, 1500 ppm M: 0, 3.7, 18.7, 56.7 mg/kg bw/day F: 0, 4.1, 21.1, 62.3 mg/kg bw/day Haematological parameters were measured once in pretrial and during weeks 6 and 12 1991 RAR B.6.3.2/09 M-146978-01-1	*Cat 1: C ≤ 10 mg/kg bw/day* *Cat 2: 10 < C ≤ 100 mg/kg bw/day*	at 1500 ppm - ↓ Hb (15 %*) in females. No effects in males. - hemosiderin deposition in the liver in both sexes (males: 3/4 mild and 1/4 moderate vs 1/4 very mild/minimal and 1/4 mild in controls; females: 1/4 mild, 1/4 moderate and 2/4 severe vs 1/4 very mild/minimal and 1/4 moderate in controls) - extramedullary hematopoiesis in the spleen in females (2/4 vs 0/4 in controls) - hypercellularity (predominantly normoblasts) in bone marrow in females (3/4 vs 0/4 in controls) - ↑ spleen weight (absolute and relative 59 %) and liver weight (absolute and relative 14 %) in females - ↑ liver weight in males (absolute 13 % and relative 11 %)
1-year oral toxicity study in dog OECD Guideline 452, GLP Beagle dog, 6/sex/group oral (diet) 0, 300, 1500 7500/5000 ppm M: 0, 9.6, 52.5, 167.7 mg/kg bw/day F: 0, 10.4, 57.4, 200.7 mg/kg bw/day Blood samples for haematological measurements were collected at pretest and weeks 13, 27 and 52 1985	*after 13 weeks:* *Cat 1: C ≤ 10 mg/kg bw/day* *Cat 2: 10 < C ≤ 100 mg/kg bw/day* *after 52 weeks:* *Cat 2: 2.5 < C ≤ 25 mg/kg bw/day* *:*	at 300 ppm <u>week 13:</u> - ↓ Hb and RBC (up to 6 - 7 %) in males and females - ↑ MetHb 143 % in males and 157 % in females compared to controls (% of Hb: 1.7 vs 0.7 and 1.8 vs 0.7 in controls) - erythropoiesis in the bone marrow (minimal to slight) males and females (1/2 and 1/2 vs no incidence in controls) - iron deposits (hemosiderin) in the liver (minimal) in males (1/2 vs 0/2 in controls)

RAR B.6.3.2/10 M-146756-01-1		week 52: - ↓ Hb and RBC (up to 9 % and 7 %) in males and females - ↑ MetHb 100 % in males and 87 % in females compared to controls (% of Hb: 1.6 vs 0.8 and 1.5 vs 0.8 in controls) - erythropoiesis in the bone marrow (minimal) in females (1/4 vs 0/4 in controls) - iron deposits (hemosiderin) in the liver (slight to moderate) in females (3/4 vs 0/4 in controls) at 1500 ppm Week 13 - ↓ Hb (up to 9 %) and RBC (up to 14 %) in males and females - ↑ MetHb 486 %** in males and 357 %* in females compared to controls (% of Hb: 4.1** vs 0.7 and 3.2* vs 0.7 in controls) - ↑ Heinz bodies (o/oo) in both sexes (59 and 51** vs no incidence in controls) - erythropoiesis in the bone marrow (minimal to moderate) in males and females (2/2 and 2/2 vs no incidence in controls) - extramedullary erythropoiesis (slight) in the spleen in females (1/2 vs 0/2 in controls) - hemosiderin deposition in the spleen in females (1/2 vs 0/2 in controls) - iron deposits (hemosiderin) in the liver (minimal to slight) in males and females (1/2 and 2/2 vs no incidence in controls) - ↑ spleen weight in males (absolute 71 % and relative up to 67 %)
80-day oral toxicity study in dog no guideline, GLP Beagle dog, 2/sex/group oral (diet) Increasing or varying dosage at 150/200/500 or 75/300/0/1500 ppm, about 20-40 days/dose level, over a total of 80 days Blood samples for hematology were collected from all animals on every weekday during the study 1991 RAR B.6.3.2/11 M-146807-01-1	*Cat 2: 10< C ≤ 110 mg/kg bw/day*	at 150/200/500 ppm and 75/300/0/1500 ppm - ↑ MetHb in both sexes at 1500 ppm and in females at 500 ppm. No effects on other RBC parameters. - congestion of the spleen in one male dog at 150/200/500 ppm and in two dogs at 75/300/0/1500 ppm (1 male and 1 female)

* = p<0.05, ** = p<0.01

M= male, F= female, RBC = red blood cell, Hct = haematocrit, Hb = hemoglobin, MetHb = methemoglobin, MCHC = mean corpuscular heamoglobin concentration, MCV = mean corpuscular volume and MCH = mean corpuscular haemoglobin

Table 27 Summary table of haematological findings in relevant long-term toxicity/carcinogenicity studies and reproductive toxicity studies

Study	Doses relevant for STOT RE classification	Effects at this dose level
24-month oral carcinogenicity study in mouse OECD Guideline 451 (1981), GLP Mice, NMRI KFM-Han 60/sex/group, oral (diet) 0, 30, 150, 750 ppm M: 0, 4.2, 22, 109 mg/kg bw/day F: 0, 5.8, 31, 145 mg/kg bw/day 1986b RAR B. 6.5/05 M-146765-01	*Cat 2: 1.25 < C ≤ 12.5 mg/kg bw/day*	at 30 ppm - ↓ Hct (13 %*) in females after 104 weeks. No changes in other haematology parameters (e.g. RBC, Hb, MetHb, Heinz bodies) - ↑ spleen weight in females (absolute 15% and relative 35-37 %) after 12 months. No change after 24 months.
24-month oral chronic toxicity/carcinogenicity study in rats OECD Guideline 453 (1981), GLP Rat, Wistar KFM-Han 70/sex/group, oral (diet) 0, 60, 300, 1500 ppm M: 0, 3.2, 15.7, 79.9 mg/kg bw/day F: 0, 3.9, 19.8, 100.5 mg/kg bw/day 1986a; Addendum 1: 2000 RAR B. 6.5/03 M-146766-01	*Cat 2: 1.25 < C ≤ 12.5 mg/kg bw/day*	at 60 ppm - ↓ Hb (8 %*) and RBC (7 %*) in males - ↓ MCH (5%*) and MCV (5%*) in females - ↑ spleen weights (absolute and relative) in females - transient ↑ of MetHb 50 %* in males compared to controls after 3 months (% of Hb: up to 1.2* vs 0.8 in controls) and 60 %* in females after 6 months (% of Hb: 0.8* vs 0.5 in controls) and 38 %* after 12 months (% of Hb:1.1* vs 0.8 in controls). No effects after 24 months.
12-month oral toxicity study in rats OECD Guideline 452 (1981), GLP Rat, Sprague-Dawley 70/sex/group, oral (diet) 0, 100, 400, 1200 ppm M: 0, 6.5, 25.2, 75.0 mg/kg bw/day F: 0, 8.0, 31.7. 97.1 mg/kg bw/day 1991a RAR B. 6.5/01 M-146979-01	*Cat 2: 2.5 < C ≤ 25 mg/kg bw/day*	at 100 and 400 ppm - ↓ Hb ((3%** in males and 4 %** in females after 26 weeks - ↓ Hct (3-4 %*) in males at 100 and 400 ppm and females (4 %*) at 400 ppm after 26 weeks - ↓ RBC in females (4 %**) at 400 ppm after 26 weeks and (5 %*) after 52 weeks - no statistically significant effects on red blood cell parameters were recorded in males at week 52 - Kupffer cell pigmentation (minimal to mild) in

		males
Two generation reproductive toxicity study OECD Guideline 416 (2001), GLP oral (diet) 0, 50, 250, 1250 ppm M : 0, 4, 21 and 100 mg/kg bw/day F : 0, 3, 18 and 102 mg/kg bw/day 2003 RAR B.6.6.1/01 M-493944-02-1	*Cat 1: C ≤ 13 mg/kg bw/day* *Cat 2: 13 < C ≤ 130 mg/kg bw/day*	at 1250 ppm - congestion of the spleen in P generation (males: 20/24 vs 10/24 in controls and females: 19/24 vs 8/24 in controls) - deposition of hemosiderin pigments in the spleen in P generation (males: 18/24 vs 2/24 in controls and females: 14/24 vs 2/24 in controls) - extramedullary hematopoiesis in the spleen in P generation (males: 5/24 vs 0/24 in controls and females: 5/24 vs 0/24 in controls)
Two generation reproductive toxicity study Non-OECD TG (US-EPA 83-4), corresponding to OECD 416, GLP oral (diet) 0, 50, 250, 1250 ppm (M: 0, 4, 20, 90 mg/kg/bw/day F: 0, 6, 30, 140 mg/kg bw/day) 1986 RAR B. 6.6.1/02 M- 146764-01-1	*Cat 2: 9 < C ≤ 90 mg/kg bw/day*	at 250 ppm and 1250 ppm - hemosiderosis and extramedullary hematopoiesis in the liver at 1250 ppm (minimal or slight) - Erythropoiesis or hemosiderosis in the spleen (slight to moderate) - erythroid hyperplasia in bone marrow in males - increased spleen weight
Embryotoxicity/Teratogenicity OECD Guideline 414 , GLP oral (intubation) 0, 10, 100, 500 mg/kg bw/day (corrected:0, 7, 70, 350 mg/kg bw/day) 1985b RAR B.6.2.2/03 M-146758-01-1	*Cat 1: C ≤ 90 mg/kg bw/day* *Cat 2: 90 < C ≤ 900 mg/kg bw/day*	at 100 and 500 mg/kg bw/day - ↑ MetHb 185 %** at 100 mg/kg bw/day (% of Hb: 3.7**) and 615 %** at 500 mg/kg bw/day (% of Hb: 3.7** and 9.3 vs 1.3 in controls) compared to controls at GD 16. - ↑ Heinz bodies (371** vs 0 ‰ in controls) at 500 mg/kg bw/day

* = p<0.05, ** = p<0.01

M= male, F= female, RBC = red blood cell, Hct = haematocrit, Hb = hemoglobin, MetHb = methemoglobin, MCHC = mean corpuscular heamoglobin concentration, MCV = mean corpuscular volume and MCH = mean corpuscular haemoglobin

Repeated dose toxicity of desmedipham by the oral route has been investigated in mice, rats and dogs. No studies were carried out via the inhalation or dermal route. There are no relevant human data available with respect to repeated dose toxicity.

Following oral administration for rats, mice and dogs in the short-term studies, the effects observed are consistent with effects pointing towards methemoglobinemia, leading to changes in RBC parameters and

slight hemolytic anemia, increased activities of the bone marrow, kidney, liver and spleen - the organs mainly involved in the turnover of red blood cells - and compensatory hematopoiesis. Effects were most clearly seen in the rat study RAR B.6.3.2/02 (1984a) and in the dog study RAR B.6.3.2/10 (1985). These findings were also supported by the results from the chronic toxicity/carcinogenicity and reproductive toxicity studies. In addition, short-term studies with the structurally and toxcicologically related substance, phenmedipham, have shown similar effects on haematopoietic system (see section 10, read-across justification).

The effects on blood are considered significant health effects. However, at the dose levels approximately equal to STOT RE 2 cut-offs haematotoxic effects were rather slight or moderate. Increases in MetHb levels (% of Hb) in blood were moderate (in rats up to 5.3 % at 1200 ppm vs 1.0 % of Hb in the controls and in dogs 4.1 % at 1500 ppm vs 0.7 % of Hb in the controls, respectively). According to Solecki et al. (2005[1]), a statistically significant increase in MetHb in rodents are considered adverse and 4% of increase in methaemoglobin in dogs is considered as threshold for adversity. It should be noted that the Solecki paper considers acute exposure to MetHb inducing substances. Reductions in haemoglobin levels were never ≥ 20 % and only moderate decreases in haemoglobin (up to 11 % at 1200 ppm in rats and 15 % in dogs at 1500 ppm) and in other red blood cell parameters were observed (i.e RBC, Hct). On the other hand, increases in formation of Heinz bodies was also seen indicating degenerated haemoglobin. MetHb is usually rapidly reduced back to Hb and, in some cases, the appearance of Heinz bodies can be considered to be a more robust indicator of MetHb formation rather than measurements of blood MetHb concentrations as Heinz bodies are more persistent than MetHb[2].

There were no premature deaths in anaemic animals and no clinical signs of hypoxia at doses relevant for STOT RE classification. The observed effects on blood parameters were not accompanied by significant clinical signs of anemia or microscopic effects like necrosis, fibrosis or chirrosis in the spleen, liver or kidney. Slight increase in haemosiderin deposits were seen in the histopathology of the kidney, liver and spleen. Some of the various findings described in the histopathology i.e increased haematopoiesis in the bone marrow, liver and spleen can be considered to be signs of adaptive and reversible compensatory changes in the blood system to the increased methemoglobin levels. The degree of severity of histopathological findings was mostly slight or moderate. More severe effects were mainly seen at higher than the dose levels which trigger on classification in category 1 or category 2.

The haemotoxic effects caused by desmedipham are considered a borderline case for classification. Strictly, applying the CLP guidance (CLP Guidance, 3.9.2.5.2, Haemotoxicity), none of these effects are considered significant or sufficiently severe for classification. However, the guidance on the application of the CLP criteria also states that in the case where multiple less severe effects with regenerative capacity were observed, the classification should apply as “Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs”

Although the severity of the haemotoxic effects represents a borderline case, multiple less severe and dose-related effects with regenerative capacity involving several organs were observed rather consistently in oral repeated dose toxicity studies in three species at the dose levels approximately equal to the STOT-RE 2 guidance values. These effects are considered sufficient for classification. This conclusion is further supported by similar toxicity profile of structurally related substance phenmedipham (see section 10, read-across justification). Therefore, classification of desmedipham for STOT-RE 2 (“H373: May cause damage

[1] Roland Solecki, Les Davies, Vicki Dellarco, Ian Dewhurst, Marcel van Raaij e, Angelika Tritscher (2005). Guidance on setting of acute reference dose (ARfD) for pesticides. Food and Chemical Toxicology, 43: 1569-1593

[2] Muller A, Jacobsen H, Healy E, McMickan S, Istace F, Blaude MN, Howden P, Fleig H, Schulte A (2006). EU Working Group on Haemolytic Anaemia. Hazard classification of chemicals inducing haemolytic anaemia: An EU regulatory perspective. Regul Toxicol Pharmacol. 45(3):229-41. Review.

to organs (blood) through prolonged or repeated oral exposure") is proposed. Since studies were conducted oral route, it is not proposed to specify a route of exposure.

Effects on thyroid gland

Following oral administration of desmedipham effects on the thyroid gland (decreased T4, increased thyroid weight and increased incidence of thyroid hyperplasia/hypertrophy) have been observed in repeated toxicity studies conducted in rats and dogs. No effects on thyroid gland were seen in mice. The following table (Table 28) summarises the key thyroid findings from relevant rat and dog studies as they relate to the guidance values for STOT RE classification.

Table 28 Summary of thyroid gland findings in relevant short-term toxicity studies

Study	Doses relevant for STOT RE	T4	T3	TSH	T4 binding capacity	Thyroid weight	Thyroid histopathology
Rats							
90-day oral toxicity study in rat 0, 6, 30,60, 300 ppm M: 0, 0.5, 2.6, 5.2, 26 mg/kg bw/day F: 0, 0.5, 2.7, 5.6, 27 mg/kg bw/day 1985 RAR B.6.3.2/03 M-146760-01-1	*Cat 1: C ≤ 10 mg/kg bw/day* *Cat 2: 10 < C ≤ 100 mg/kg bw/day*	↓ ♀♂	na	nd	nd	na	na
90-day oral toxicity study in rat 0, 160, 800, 4000 ppm M: 0, 10.6, 54, 275 mg/kg bw/day F: 0, 12.3, 60, 339 mg/kg bw/day 1987 RAR B.6.3.2/04 M-146976-01-1	*Cat 2: 10 < C ≤ 100 mg/kg bw/day*	nd	nd	nd	nd	na	↑ follicular hyperthophy (minimal to moderate) ♀♂ at 800 ppm. Incidences of 0/10, 1/10 and 4/10 in males; 0/10, 0/10 and 3/10 in females in the control, 160 ppm and 800 ppm groups, respectively
90-day oral toxicity study in rat 0, 300, 1200, 4800 ppm M: 0, 24, 97, 415 mg/kg bw/day F: 0, 27, 109, 378 mg/kg bw/day 1984a RAR B.6.3.2/02 M-146746-01-1	*Cat 2: 10 < C ≤ 100 mg/kg bw/day*	nd	nd	nd	nd	na	↑ follicular hyperplasia (minimal to slight) ♀♂ at 1200 ppm Incidences of 0/10, 1/10 and 8/10 in males; 0/10, 0/10 and 10/10 in females in the control, 300 ppm and 1200 ppm groups, respectively
Dogs							
90-day oral toxicity study in dog 0, 1, 5, 150 ppm M: 0.035, 0.17 or 4.97, mg/kg/day F: 0.035, 0.19 or 5.50	*Cat 1: C ≤ 10 mg/kg bw/day*	↑♂	na	↓♀↑♂	↓ ♀♂	na	na

mg/kg/day for both M/F: 0, 0.035, 0.18, 5.24 mg/kg bw/day 1986 RAR B.6.3.2/08 M-146762-01-1							
1-year oral toxicity study in dog 0, 300, 1500 7500/5000 ppm M: 0, 9.6, 52.5, 167.7 mg/kg bw/day F: 0, 10.4, 57.4, 200.7 mg/kg bw/day 1985 RAR B.6.3.2/10 M-146756-01-1	<u>Week 13</u>: *Cat 2: 10 < C ≤ 100 mg/kg bw/day* <u>Week 52:</u> *Cat 2: 2.5 < C ≤ 25 mg/kg bw/day*	↓ ♀	↓ ♀♂	nd	nd	↑♀♂ at 1500 and 5000 ppm after 13 weeks at 1500 ppm in males absolute 57 %**, relative to bw 44 %* and relative to brain 52%*; in females: absolute 105 %**, relative to bw 100 %** and relative to brain 87 %**	↑follicular hyperplasia ♂♀ at 1500 ppm (minimal to slight) after 13 weeks. Incidences of 0/4, 0/4 and 4/4 after 13 weeks and in 0/8, 0/8, 4/8 and after 52 weeks of treatment at 0, 300 and 1500 ppm, respectively. (At study termination, follicular hyperplasia in thyroids was noted only in the 1500 ppm female group)
90-day oral toxicity study in dog 0, 100, 500, 1500 ppm M: 0, 3.7, 18.7, 56.7 mg/kg bw/day F: 0, 4.1, 21.1, 62.3 mg/kg bw/day 1991 RAR B.6.3.2/09 M-146978-01-1	*Cat 1: C ≤ 10 mg/kg bw/day* *Cat 2: 10 < C ≤ 100 mg/kg bw/day*	nd	nd	nd	nd	↑♀(absolute and relative 38%**) at 1500 ppm	↑ follicular hypertrophy (minimal to mild) ♀at 500 and 1500 ppm; ♂at 1500 ppm Incidences of 0/4, 4/4* and 4/4* in females in control, 500 and 1500 ppm groups; 0/4 ,2/4 and 4/4* in males in control, 500 ppm and 1500 ppm groups

* = p<0.05, ** = p<0.01

Na= not affected, Nd=not determined, ♀=females, ♂=males, ↓=decrease, ↑ increase

In a 13-week dietary study (1985, RAR B.6.3.2/03), at dose levels relevant for STOT RE pituitary weights (absolute, relative to brain weight ratios) were significantly reduced in 300 ppm (13 % for both) and in females at 60 ppm (relative 17%) and 300 ppm (relative weights were not affected. T4 levels were decreased significantly in both sexes at 300 ppm and 300 ppm during the recovery period (see

Table 29). At the dose level of 60 ppm absolute and relative relative liver weights (11 %) were decreased in females.

Table 29 Mean values of T3 and T4 and chloride in males and females (1985, RAR B.6.3.2/03)

Males

	CHLORIDE mmol/l	T3 nmol/l	T4 nmol/l
4/5 WEEKS			
GROUP 1	109.6	0.67	66.2
GROUP 2	110.8	0.55	62.6
GROUP 3	110.9	0.56	60.6
GROUP 4	109.5	0.58	56.6 *
GROUP 5	111.4 *	0.69	60.4
12/13 WEEKS			
GROUP 1	112.8	1.20	35.4
GROUP 2	113.3	1.14	32.0
GROUP 3	113.8	1.11	31.4
GROUP 4	114.2	1.25	30.7
GROUP 5	114.0	1.33	24.8 *
16/17 WEEKS			
GROUP 1	109.9	1.05	33.4
GROUP 2	111.3	0.95	30.2
GROUP 3	111.4 *	0.88	27.3
GROUP 4	112.0 *	0.74 *	27.9
GROUP 5	111.9 *	0.83	25.6 *

females

	CHLORIDE mmol/l	T3 nmol/l	T4 nmol/l
4/5 WEEKS			
GROUP 1	111.7	0.72	52.0
GROUP 2	112.3	0.77	50.5
GROUP 3	111.7	0.82	55.2
GROUP 4	110.7	0.80	54.8
GROUP 5	109.5	0.74	45.8
12/13 WEEKS			
GROUP 1	114.7	1.27	21.3
GROUP 2	114.9	1.25	17.1
GROUP 3	115.2	1.34	23.9
GROUP 4	114.6	1.36	18.5
GROUP 5	116.1	1.08	6.6 *
16/17 WEEKS			
GROUP 1	113.3	0.87	18.0
GROUP 2	112.5	0.80	15.2
GROUP 3	113.1	0.75	14.4
GROUP 4	111.2 *	0.75	14.6
GROUP 5	112.3	0.68	11.7

Dose levels 0 (Group 1), 6 (Group 2), 30 (Group 3), 60 (Group 4) and 300 (Group 5) ppm

* = $p<0.05$, ** = $p<0.01$

In a 13-week dietary study (1987, RAR B.6.3.2/04), relative thyroid weights in males and absolute thyroid weights in females were statistically significantly increased at 4000 ppm. Absolute weights of pituitary gland were significantly lower in females at 4000 ppm than in the control group (15%). At dose levels relevant for STOT RE 2 classification (≤ 800 ppm) no significant changes in thyroid gland weight was seen but minimal to moderate follicular cell hypertrophy was seen in both sexes with low incidence of 4/10 in males and 3/10 in females at 800 ppm (0/10 in controls). Levels of T4, T3 or TSH were not determined.

In a 13-week dietary study (1984a, RAR B.6.3.2/02), an increased incidence in thyroid follicular cell hyperplasia was observed in both males and females at 1200 ppm and 4800 ppm. At dose levels relevant for STOT RE 2 classification (≤ 1200 ppm) the incidence of follicular cell hyperplasia was 8/10 in males and 10/10 in females at 1200 ppm (0/10 in controls). The severity of observed hyperplasia was graded as minimal to slight. Levels of T4, T3 or TSH were not determined. Thyroid weights were not affected. At the dose level of 1200 ppm relative liver weights were increased (18 %) in females.

In a 1-year study (1985, RAR B.6.3.2/10), increased follicular cell hypertrophy was observed in males and females at 1500 and 7500/5000 ppm after 3 months. After 24 months follicular cell hyperplasia was seen in females at 1500 ppm and in both sexes at 7500/5000 ppm. At dose levels relevant for STOT RE 2 classification (≤ 1500 ppm), the incidence of follicular cell hypertrophy after 3 months administration was 0/4 and 4/4 in the control and 1500 groups, respectively. Number of animals examined was very low (2 dogs/sex/group). The severity of hypertrofia was graded as a minimal to slight. Increased absolute and relative thyroid weights were observed in males and females at 1500 and 5000 ppm after 3 months. After 24 months, the absolute and relative thyroid weights were increased in females at 1500 ppm and 5000 ppm and

in males at 5000 ppm. The levels of T4 were decreased significantly in females at 1500 and 5000/7500 ppm and T3 in all dose levels (see Table 30). In males T3 levels were decreased at 300 ppm and 1500 ppm but not at the highest dose level (no statistical significance). TSH levels were not determined. Relative liver weights were increased at 1500 ppm in males (no statistical significance, absolute 71 % and relative up to 67 %) and females after 52-week exposure.

Table 30 Clinical biochemistry (1985, RAR B.6.3.2/10)

Males

	T3 nmol/l	T4 nmol/l
PRETEST		
GROUP 1	---	---
GROUP 2	---	---
GROUP 3	---	---
GROUP 4	---	---
AT 13 WEEKS		
GROUP 1	---	---
GROUP 2	---	---
GROUP 3	---	---
GROUP 4	---	---
AT 27 WEEKS		
GROUP 1	---	---
GROUP 2	---	---
GROUP 3	---	---
GROUP 4	---	---
AT 52 WEEKS		
GROUP 1	1.59	37.8
GROUP 2	1.19 *	37.8
GROUP 3	0.99 **	30.2
GROUP 4	1.44	26.1

Females

	T3 nmol/l	T4 nmol/l
PRETEST		
GROUP 1	---	---
GROUP 2	---	---
GROUP 3	---	---
GROUP 4	---	---
AT 13 WEEKS		
GROUP 1	---	---
GROUP 2	---	---
GROUP 3	---	---
GROUP 4	---	---
AT 27 WEEKS		
GROUP 1	---	---
GROUP 2	---	---
GROUP 3	---	---
GROUP 4	---	---
AT 52 WEEKS		
GROUP 1	1.74	50.6
GROUP 2	1.29 *	40.1
GROUP 3	1.32 *	31.0 **
GROUP 4	0.88 **	29.0 **

* = $p<0.05$, ** = $p<0.01$

Group 1: 0 ppm, group2: 300 ppm, group 3:1500 ppm and group 4: 7500/5000 ppm.

In a 90-day study in dogs (1986, RAR B.6.3.2/08), pituitary cysts were noted in female dogs (3/4) at the dose level above (150 ppm) cut-offs for STOT RE 2 classification. This finding was detected histopathological finding was not confirmed by other studies in dog. Transient changes in levels of thyroxine in blood were also observed. The level of T4 was increased in males at 5 ppm, TSH levels were decreased in females and increased in males at 5 ppm and T4 binding capacity was significantly reduced in females at 5 ppm and in males at 1 ppm when compared to controls (see Table 31 and Table 32). No effects on thyroid weights or histopathological changes in the thyroid were reported. No effects on liver weights were seen.

Table 31 Group mean clinical chemistry values in male dogs day 30 and 58 (1986, RAR B.6.3.2/08)

Group Mean Clinical Chemistry Values.
MALE DOGS - DAY 30

Parameter		DESMEDIPHAM ppm 0	1	5	150
THYROXINE nmol/l	X (SD)	37 (4)	39 (5)	38 (3)	45* (6)
TOTAL T3 nmol/l	X (SD)	1.3 (0.2)	1.1 (0.3)	1.3 (0.1)	1.5 (0.3)
T4-BINDING CAPACITY TBI	X (SD)	0.92 (0.04)	0.88 (0.07)	0.88 (0.06)	0.87 (0.06)
FREE T4 INDEX FT4I	X (SD)	41 (5)	45 (7)	43 (4)	51* (7)

p = < 0.001 = ***
p = < 0.01 = **
p = < 0.05 = *

Group Mean Clinical Chemistry Values.
MALE DOGS - DAY 58

Parameter		DESMEDIPHAM ppm 0	1	5	150
THYROXINE nmol/l	X (SD)	38 (6)	37 (3)	39 (9)	41 (5)
TOTAL T3 nmol/l	X (SD)	1.2 (0.4)	1.4 (0.4)	1.6 (0.3)	1.5 (0.2)
T4-BINDING CAPACITY TBI	X (SD)	0.87 (0.02)	0.85 (0.02)	0.80** (0.04)	0.80** (0.01)
FREE T4 INDEX FT4I	X (SD)	43 (6)	43 (3)	49 (10)	51 (6)

p = < 0.001 = ***
p = < 0.01 = **
p = < 0.05 = *

Table 32 Group mean clinical chemistry values in female dogs day 59 and 86 (1986, RAR B.6.3.2/08)

Group Mean Clinical Chemistry Values.
FEMALE DOGS - DAY 58

Parameter		DESMEDIPHAM ppm 0	1	5	150
THYROXINE nmol/l	X (SD)	43 (7)	37 (5)	41 (3)	34* (6)
TOTAL T3 nmol/l	X (SD)	1.6 (0.3)	1.4 (0.1)	1.6 (0.3)	1.4 (0.4)
T4-BINDING CAPACITY TBI	X (SD)	0.88 (0.10)	0.80 (0.05)	0.84 (0.04)	0.79 (0.04)
FREE T4 INDEX FT4I	X (SD)	50 (11)	46 (5)	49 (6)	43 (8)

p = < 0.001 = ***
p = < 0.01 = **
p = < 0.05 = *

Group Mean Clinical Chemistry Values.
FEMALE DOGS - DAY 86

Parameter		DESMEDIPHAM ppm 0	1	5	150
THYROXINE nmol/l	X (SD)	42 (8)	34 (3)	34 (4)	31* (9)
TOTAL T3 nmol/l	X (SD)	1.9 (0.3)	1.7 (0.2)	1.8 (0.2)	1.7 (0.2)
T4-BINDING CAPACITY TBI	X (SD)	0.67 (0.12)	0.68 (0.04)	0.69 (0.08)	0.62 (0.05)
FREE T4 INDEX FT4I	X (SD)	64 (18)	51 (7)	51 (11)	49 (13)

p = < 0.001 = ***
p = < 0.01 = **
p = < 0.05 = *

In a 90-day study in dogs (1991, RAR B.6.3.2/09), at the doses relevant for STOT RE classification, the incidence of follicular cell hypertrophy was increased in females and males (0/4, 4/4 and 4/4; 0/4, 2/4 and 4/4 at 0, 500 and 1500 ppm, respectively). The severity of hypertrophy was graded as minimal/mild. Thyroid glands were increased in weight at 1500 ppm in females and absolute pituitary weights were increased in males at 500 ppm (29%). Levels of T4, T3 or TSH were not determined. Slightly increased liver weight (no statistical significance) was seen in males and females at 500 ppm and 1500 ppm.

In the two generation toxicity study (dRAR B.6.6.1/02, 1986) slight to moderate thyroid follicular hyperplasia in thyroid were observed in parental animals in F1 generation at 250 ppm (20-30 mg/kg/bw/day) and 1250 ppm (90 - 140 mg/kg bw/day). Effects on the thyroid gland were also seen in the long-term toxicity and carcinogenicity studies in rats and mice. However, the effects were seen clearly above the dose levels relevant for STOT RE 2 classification.

The effects on thyroid system in available repeated dose toxicity studies (increased thyroid weight, follicular hypertrophy, decreased T3 and T4) did not always occur at doses which also caused signs of liver toxicity (1987, RAR B.6.3.2/04; 1991, RAR B.6.3.2/09).

Induction of liver enzymes in rats after oral treatment with desmedipham were examined in a 28-day toxicity study in the rat by dietary administration (2014, RAR B.6.8.2/01). Desmedipham treatment up-regulated Ugt2b1 transcript from the UDP glucuronosyltransferase gene family in rats indicating liver enzyme induction. UDP-glucuronosyltransferase (UGT) induction in liver, Cytochrome P450 (CYP) and UGT mRNA expression levels were measured in an in vitro liver model, i.e. DMP-treated dog hepatocytes (2017, RAR B.6.8.2/02). Desmedipham treatment up-regulated CYP2B6 and CYP3A4, and also UGT2B31 mRNA in dog hepatocytes like phenobarbital. Desmedipham was negative in four thyroid receptor alpha-related assays in ToxCast (see further RAR Vol 3. B.6, Appendix 2).

Overall, in conclusion, the thyroid effects observed in oral repeated dose toxicity studies at the dose levels relevant for STOT RE 2 classification are not considered sufficiently severe or marked enough for justify classification. Therefore, no classification for effects on thyroid gland is proposed.

Effects on acetylcholinesterase activity

Acetylcholinesterase was measured in four repeated dose studies in rats and in two studies in dogs.

In a 13-week dietary study (1984a, RAR B.6.3.2/02), the doses relevant for STOT-RE 2 classification are at or below 1200 ppm. The author concluded that plasma cholinesterase activity was lower by approximately 30% in females at 1200 ppm and by approximately 60% in females at 4800 ppm (RAR). This finding was considered to be dose-related. Brain acetyl cholinesterase activity was decreased by approximately 20% at both 1200 and 4800 ppm in females (Table 33)

The variability of the cholinesterase levels (greater than 20%) was considered to be due in part to the limited number of cholinesterase assays conducted within the laboratory performing the test (RAR). In the report (see section 5.3.1.4.3 RAR B.6.3.2/03), clinical biochemistry reference values are presented for clinical chemistry data collected between the period of June 1981 to October 1984 (Table 34). These data were exclusively compiled from this study which was conducted between January and April 1983. No cholinesterase assays had therefore been performed in the rat for at least two years prior to this study. It is suggested that the laboratory had limited contemporary experience at conducting the assays. Therefore, since the level of brain cholinesterase, the critical end-point (given that plasma cholinesterase inhibition is a poor marker of adverse effects in man), was only 20% lower than control values it considered to fall within the range of possible variation for the laboratory. Therefore, it has been concluded that there is no evidence of a conclusive effect on this parameter. Particularly given the lack of concordance with the red cell cholinesterase levels.

Table 33 Cholinesterase measurements in the 13 week rat study (1984a, RAR B.6.3.2/02)

Parameter		Dose Level (ppm)							
		Males				Females			
		0	300	1200	4800	0	300	1200	4800
Test material intake (mg/kg/day)		0	24	97	415	0	27	109	378
Week 13	BuChE Plasma	0.37	0.36	0.40	0.41	1.65	1.45	1.17**	0.68**
	(% control)	-	(97%)	(108%)	(111%)	-	(88%)	(71%)	(41%)
	AchE RBC	2.25	2.59**	2.77**	2.59**	2.12	2.21	2.46**	2.54**
	(% control)	-	(115%)	(123%)	(115%)	-	(104%)	(116%)	(120%)
	AchE Brain	4.59	5.32**	4.48	3.78**	7.38	6.56*	5.77**	5.94**
	(% control)	-	(116%)	(98%)	(82%)	-	(89%)	(78%)	(81%)

*p ≤ 0.05, **p ≤ 0.01

Method : Acetylthiocholine iodide/DTNB – Technicon Autoanalyser II continuous flow system

Key: BuChE = plasma cholinesterase and AchE RBC = red blood cell cholinesterase AchE Brain = brain cholinesterase

Table 34 Historical control cholinesterase values from RAR B.6.3.2/03 (1985)

Parameter	Age	Males				Females			
		Mean	S.D.	Min	Max	Mean	S.D.	Min	Max
Bu ChE - Plasma	7-9 weeks	0.44	0.04	0.38	0.48	1.11	0.17	0.84	1.30
	13-18 weeks	0.37	0.07	0.27	0.50	1.65	0.33	1.18	2.22
AchE - Ery	7-9 weeks	1.77	0.04	1.73	1.81	2.15	0.13	1.97	2.33
	13-18 weeks	2.25	0.24	1.80	2.65	2.12	0.31	1.71	2.65
AchE-Brain	7-9 weeks	4.56	0.60	3.56	5.06	4.20	0.18	3.99	4.42
	13-18 weeks	4.59	0.43	3.86	5.36	7.38	0.89	6.43	9.01

In a 13-week dietary study (1987, RAR B.6.3.2/04), the doses relevant for STOT-RE 2 classification are at or below 800 ppm. The author concluded that plasma cholinesterase levels for male rats at 4000 ppm was higher than controls (RAR). For females, plasma cholinesterase levels were generally lower than those of controls. Similarly, lower brain cholinesterase levels were recorded at the end of the study for males receiving 800 ppm (20 %) and 4000 ppm (21 %). Brain cholinesterase levels for female rats were similar to controls and no changes in erythrocyte cholinesterase levels were observed in either sex.

As with the Suter study there is clearly a high level of variability in the assays (RAR). Given this, the limited differences with controls, the lack of concordance between brain, red cell and plasma cholinesterase there is no evidence of a clear effect on cholinesterase in this study.

Table 35 Cholinesterase measurements in the 13 week rat study (1987, RAR B.6.3.2/04)

Parameter		Dose Level (ppm)							
		Males				Females			
		0	160	800	4000	0	160	800	4000
Test material intake (mg/kg/day)		0	10.6	54	275	0	12.3	60	339
Week 13	Plasma ChE	0.45	0.50	0.54	0.57*	2.06	1.96	1.91	1.64
	(% control)	-	(111%)	(120%)	(127%)	-	(95%)	(93%)	(80%)
	AChE RBC	1.74	1.59	1.85	1.80	1.85	1.92	2.02	1.95
	(% control)	-	(91%)	(106%)	(103%)	-	(104%)	(92%)	(105%)
	AChE Brain	7.95	7.53	6.39**	6.26**	6.55	6.53	6.85	6.54
	(% control)	-	(95%)	(80%)	(79%)	-	(100%)	(105%)	(100%)

*$p \leq 0.05$, **$p \leq 0.01$

Method : Ellman, G. L. et al , Biochem. Pharm., 1961, Vol 7, pp 88-95.
Plasma ChE = plasma cholinesterase and AChE RBC = red blood cell cholinesterase and AchE Brain = brain cholinesterase

In a 52-week dietary study (1991a, RAR B. 6.5/01), the doses relevant for STOT-RE 2 classification are at or below 400 ppm. The only statistically significant differences seen between test and control groups were a lower level of plasma cholinesterase (27%) in females at the high dose level of 1200 ppm after 26 weeks, and a higher level of plasma cholinesterase (36%) in females at the low dose level of 100 ppm after 52 weeks. No statistically significant differences were seen in either sex for red cell cholinesterase after either 26 or 52 weeks

or on brain cholinesterase after 52 weeks. Taking into consideration the inherent variability of the levels of plasma cholinesterase it have been concluded that there was no clear effect on cholinesterase in either sex at any of the dose levels tested. In conclusion, no significant differences in cholinesterase levels were seen at the dose levels (≤ 400 ppm) relevant for STOT-RE 2 classification.

Table 36 Cholinesterase measurments in the 52 week rat study (1991a, RAR B. 6.5/01)

Parameter		Dose Level (ppm)							
		Males				Females			
		0	100	400	1200	0	100	400	1200
Test material intake (mg/kg/day)		0	6.5	25.2	75.0	0	8.0	31.7	97.1
Week 26	Plasma ChE	562	578	663	665	2793	3072	2578	2041*
	(% control)	-	(102%)	(118%)	(118%)	-	(110%)	(92%)	(73%)
	AChE RBC	489	507	513	496	656	720	834	741
	(% control)	-	(104%)	(95%)	(101%)	-	(110%)	(127%)	(113%)
Week 52	Plasma ChE	721	757	875	953	2383	3248*	2502	2094
	(% control)	-	(105%)	(121%)	(132%)	-	(136%)	(105%)	(88%)
	AChE RBC	479	508	559	536	797	1064	1049	634
	(% control)	-	(106%)	(117%)	(112%)	-	(134%)	(132%)	(80%)
	AchE Brain	14768	15507	14821	14375	13058	15113	14746	14190
	(% control)	-	(105%)	(100%)	(103%)	-	(116%)	(113%)	(109%)

*p ≤ 0.05, **p ≤ 0.01

Method : Ellman, G. L. et al , Biochem. Pharm., 1961, Vol 7, pp 88-95 for red cell and brain cholinesterase
Pilz,W., Johann, I. And Stelzl, E., Z. Anal. Chem. 215, 260, (1966). For plasma cholinesterase
Plasma ChE = plasma cholinesterase and AChE RBC = red blood cell cholinesterase and AchE Brain = brain cholinesterase

In a 104-week dietary study (1991b, RAR B. 6.5/02), no significant differences in cholinesterase level were seen at the dose level 100 ppm which is relevant for STOT-RE 2 classification. No statistically significant differences in the levels of plasma or red cell cholinesterase after 79 and 104 weeks of treatment or in the level of brain cholinesterase at 104 weeks were detected. It is therefore concluded that there was no evidence that desmedipham had an effect on cholinesterase in this study at a dose level of up to 64 to 86 mg/kg/day.

Table 37 Cholinesterase measurments in the 104-week rat study (1991b, RAR B. 6.5/02)

Parameter		Dose Level (ppm)							
		Males				Females			
		0	100	400	1200	0	100	400	1200
Test material intake (mg/kg/day)		0	5.4	21.6	64.4	0	6.9	28.4	86.5
Week 79	Plasma ChE	922	916	1186	1210	2408	2419	2445	2105
	(% control)	-	(99%)	(129%)	(131%)	-	(100%)	(102%)	(87%)
	AChE RBC	1653	1204	1135	1133	988	979	1097	1003
	(% control)	-	(73%)	(69%)	(69%)	-	(99%)	(111%)	(102%)

Week 104	Plasma ChE	1030	894	1118	1077	2058	1993	1692	1585
	(% control)	-	(87%)	(109%)	(105%)	-	(97%)	(82%)	(77%)
	AChE RBC	422	383	486	404	493	492	431	461
	(% control)	-	(91%)	(115%)	(96%)	-	(100%)	(87%)	(94%)
	AchE Brain	13780	13237	12509	9639	14215	12531	11705	12823
	(% control)	-	(96%)	(91%)	(70%)	-	(88%)	(82%)	(90%)

Method : Ellman, G. L. et al , Biochem. Pharm., 1961, Vol 7, pp 88-95 for red cell and brain cholinesterase
Pilz,W., Johann, I. And Stelzl, E., Z. Anal. Chem. 215, 260, (1966). For plasma cholinesterase
Plasma ChE = plasma cholinesterase and AChE RBC = red blood cell cholinesterase and AchE Brain = brain cholinesterase

In a 28-day dose-range finding study (1984, RAR B.6.3.2/06), the doses relevant for STOT-RE classification are at or below 5000/7000 ppm. The high dose level was increased after 3 weeks from 5000 to 7000 ppm. The author concluded that there were no treatment-related effects on plasma, red cell or brain cholinesterase as all the results were within the normal range. Comparison of plasma and red cell cholinesterase levels at 4 weeks with the pre-exposure values showed there was no effect of treatment. However, it should be noted that the pre-test level of red cell cholinesterase for dog number 7 was extremely low and that the post-exposure level of red cell cholinesterase for dog number 6 was also very low. This indicated the variability of the cholinesterase assays within the laboratory conducting the study (RAR).

Table 38 Cholinesterase measurements in the 28 day range finding study in dog (1984, RAR B.6.3.2/06)

Parameter		Dose Level (ppm)							
		Males				Females			
		0	200	1000	5/7000	0	200	1000	5/7000
Dog Number		1	2	3	4	5	6	7	8
Test material intake (mg/kg/day)		0	7	30	151-214	0	7	30	151-214
Pre-test	BuChE Plasma	5.99	5.99	6.16	5.47	6.67	5.99	4.62	6.50
	AChE RBC	3.33	1.97	2.57	1.88	3.25	3.42	1.28	2.74
4 weeks	BuChE Plasma	5.64	5.81	5.56	5.47	5.81	5.39	4.45	6.95
	(% pre-test)	(94%)	(97%)	(90%)	(100%)	(87%)	(90%)	(96%)	(107%)
	AChE RBC	3.33	2.05	2.82	2.22	3.42	1.45	3.93	2.99
	(% pre-test)	(100%)	(104%)	(110%)	(118%)	(105%)	(42%)	(307%)	(109%)
	AChE Brain	5.15	7.29	7.08	4.08	4.29	5.58	4.29	5.58
	(% control)	-	(142%)	(137%)	(79%)	-	(130%)	(100%)	(130%)

Method : Acetylthiocholine iodide/DTNB – Technicon Autoanalyser II continuous flow system

Key: BuChE = plasma cholinesterase and AchE RBC = red blood cell cholinesterase AchE Brain = brain cholinesterase

In a 1-year study in dogs (1985, RAR B.6.3.2/10), the doses relevant for STOT-RE classification are at or below 1500 ppm (after 13 weeks). Eight dogs at 5000 ppm and one animal in each of the 1500 and 300 ppm groups showed occasional signs of decreased activity, tremors, ataxia, spasms and episodes of lateral or ventral recumbency which persisted for 2 - 20 minutes. No effects were seen on plasma or red cell cholinesterase after 13, 27 or 52 weeks of treatment. In addition, there was no effect on brain cholinesterase after 52 weeks.

Table 39 Cholinesterase measurments in the 12-month oral toxicity study in dog (1985, RAR B.6.3.2/10)

Parameter		Dose Level (ppm)							
		Males				Females			
		0	300	1500	7500 /5000	0	300	1500	7500 /5000
Test material intake (mg/kg/day)		0	9.8	53	241/171	0	9.8	53	241/171
Pre-test	BuChE Plasma	6.11	5.73	6.70	5.27	5.63	5.66	5.46	5.30
	(% control)	-	(94%)	(110%)	(86%)	-	(101%)	(97%)	(94%)
	AChE RBC	2.95	2.51	3.35	2.91	3.16	3.23	3.06	3.15
	(% control)	-	(85%)	(114%)	(99%)	-	(102%)	(97%)	(100%)
13 weeks	BuChE Plasma	7.17	6.98	7.72	6.87	7.25	7.47	7.22	7.92
	(% control)	-	(97%)	(108%)	(96%)	-	(103%)	(100%)	(109%)
	AChE RBC	3.43	2.86	3.90	3.08	3.38	3.82	3.58	3.06
	(% control)	-	(83%)	(114%)	(90%)	-	(113%)	(106%)	(91%)
	AchE Brain	4.83	4.50	5.15	3.86	4.93	6.11	5.26	7.94
	(% control)	-	(93%)	(107%)	(80%)	-	(122%)	(107%)	(161%)

27 weeks	BuChE Plasma	4.70	5.32	5.19	4.92	6.60	6.33	5.64	5.50
	(% control)	-	(113%)	(110%)	(105%)	-	(96%)	(85%)	(83%)
	AChE RBC	2.63	2.31	3.06	2.44	2.33	3.51	3.12	2.76
	(% control)	-	(88%)	(116%)	(93%)	-	(151%)	(134%)	(118%)
52 weeks	BuChE Plasma	6.75	7.46	7.40	7.81	8.27	7.46	7.14	8.93
	(% control)	-	(111%)	(110%)	(116%)	-	(90%)	(86%)	(108%)
	AChE RBC	3.18	2.91	3.63	2.99	2.95	4.23	3.66	4.40
	(% control)	-	(92%)	(114%)	(94%)	-	(143%)	(124%)	(149%)
	AchE Brain	4.02	4.24	4.34	4.29	4.77	4.08	6.22	6.65
	(% control)	-	(105%)	(108%)	(107%)	-	(86%)	(130%)	(139%)

Method : Acetylthiocholine iodide/DTNB – Technicon Autoanalyser II continuous flow system

Key: BuChE = plasma cholinesterase and AchE RBC = red blood cell cholinesterase AchE Brain = brain cholinesterase

Overall, in conclusion, at the doses relevant for STOT-RE 2 classification (≤ 1200 ppm), some decreases in plasma and brain cholinesterase were noted in the two rat subchronic studies. However, given the small magnitude of the difference in brain cholinesterase, the considerable variability of the results in the assays (e.g. not consistent in direction and sex) and no inhibition of red cell cholinesterase, these differences were not considered to be toxicologically significant. The effects on cholinesterase observed in oral repeated dose toxicity studies at the dose levels relevant for STOT RE 2 classification are not considered sufficient for classification.

10.12.3 Conclusion on classification and labelling for STOT RE

Based on haemotoxic effects seen in repeated dose toxicity studies in mice, rats and dogs, classification of desmedipham for STOT-RE 2 ("H373: May cause damage to organs (blood) through prolonged or repeated oral exposure") is proposed. Since studies were conducted oral route, it is not proposed to specify a route of exposure. No specific concentration limit for haemotoxic effects is proposed.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The repeat dose toxicity of desmedipham via the oral route has been investigated in the rat, mouse and dog. The following effects are discussed in the CLH report:

- Haemolytic anaemia was observed in the rat, mouse and dog. Although the effects were below the guidance values (guidance values) and did not meet any of the individual criteria listed in the CLP guidance (v. 5.0), the DS proposed classification in Category 2 based on "generalised changes of a less severe nature involving several organs".

Nevertheless, the DS indicated this to be a borderline case between Category 2 and no classification.

- . The DS did not consider thyroid-related effects in the rat and the dog sufficiently severe to warrant classification.
- . The DS did not consider the finding of reduced acetylcholinesterase (AChE) activity in the rat sufficient for classification due to the small magnitude of the brain AChE reduction, considerable variability (indicated by the lack of a dose-response relationship and lack of consistency between sexes) and the absence of an effect on erythrocyte AChE.

Overall, the DS proposed classification with STOT RE 2; H373 (blood).

Comments received during public consultation

Comments were provided by 4 Member State Competent Authorities (MSCAs) and 1 Industry association.

Three MSCAs supported classification as STOT RE 2 (blood). One of them proposed to additionally consider classification for the following organs:

- Lungs, due to the increased inflammatory reaction seen in the lungs of rats (B.6.5/03) and mice (B.6.5/05)
- Ovary, due to the increased incidences of ovarian cysts in rats (B.6.5/03) and mice (B.6.5/05)
- Liver, due to "the clear liver toxicity evident in the long-term studies"

In response, the DS analysed the effects in the lungs, ovaries and liver in the long-term rat and mouse studies and concluded that no clear effects on these organs were seen at dose levels relevant for STOT RE 2 classification.

One MSCA agreed with the DS that the haematotoxicity classification might be a borderline case but noted that none of the individual studies fulfils the classification criteria. Regarding the DS's proposal to take into account "generalised changes of a less severe nature involving several organs", the MSCA pointed out that the various adverse effects concern specific effects (i.e. effects on the haematological system) and not generalised changes involving several organs.

An industry association also disagreed with the haematotoxicity classification but focused in their argumentation on the relatively week effects at 300 ppm in the 90-day rat study 6.3.2/03. As to the thyroid effects, they provided an analysis supporting a rodent-specific mode of action via liver enzyme induction and pointed out the weak potency evidenced by lack of thyroid tumours in the rat carcinogenicity studies.

Assessment and comparison with the classification criteria

RAC identified the following effects potentially relevant for the STOT RE classification in the available studies with desmedipham:

- Haemolytic anaemia

- Thyroid-related findings
- Reduced AChE activity

These effects are discussed below. RAC agrees with the DS that no effects were observed in other organs at doses below the guidance values for classification.

Haematotoxicity

Effects indicative of regenerative haemolytic anaemia (such as reduced erythrocyte count, reduced haemoglobin (Hb), increased methaemoglobin (MetHb), presence of Heinz bodies, enlarged spleen, haemosiderin deposition in the spleen, liver and kidney, increased reticulocytes, increased extramedullary haematopoiesis) were seen across studies and species. A detailed summary of effects below the guidance values (extrapolated according to Haber's rule) is provided in Tables 26 and 27 of the CLH report. Additional information can be found under 'Supplemental information' and in the RAR.

Haematological effects have been observed in studies of various durations, from 10-day PNDT studies to 2-year studies. The CLP regulation provides guidance values for 90-day studies. For studies of a different duration, guidance values can be extrapolated using Haber's rule. Haber's rule says that the product of effective concentration (or dose) and exposure time is constant. Haematological measurements in study B.6.5/03 show that the effective doses for Hb reduction and MetHb increase are the same regardless of whether exposure duration is 3 months or 2 years. Comparison of studies B.6.3.2/02 and B.6.6.2/03 shows that the degree of methaemoglobinaemia after 3 months is similar to that after 10 days of exposure (see 'Supplemental information'). This information indicates that the effective dose for haematotoxic effects of desmedipham does not decrease with time. In other words, the effect does not follow Haber's rule. Therefore, RAC does not consider extrapolation of guidance values using Haber's rule appropriate in this particular case and the default guidance value of 100 mg/kg bw/d will be used in the assessment. In addition, studies of longer duration will be given more weight in the assessment than short-term studies (in line with CLP guidance, 3.9.2.3.2).

Specific guidance on classification of substances causing haemolytic anaemia is available, according to which, if a haemolytic substance induces one or more serious health effects listed in the table below within the critical range of doses, classification is warranted. It is sufficient for classification that only one of these criteria is fulfilled. The table summarises the effects in studies with desmedipham corresponding to the individual criteria.

Comparison of the haematotoxicity-related findings with the criteria of the CLP guidance

Criterion	**Corresponding effects in studies with desmedipham**	**Reference(s)**
(1) Premature deaths in anaemic animals that are not limited to the first three days of treatment in the repeated dose study	None	–
(2) Clinical signs of hypoxia, e.g. cyanosis, dyspnoea, pallor, in anaemic animals that are not limited to the first three days of treatment in the repeated dose study	Pallor in one 90-day study at ca. 300 mg/kg bw/d	90-day rat study B.6.3.2/04, 4 000 ppm
(3) Reduction in Hb at ≥ 20 %	Maximum Hb reduction	90-day rat study

(4) Reduction in functional Hb at ≥ 20 % due to a combination of Hb reduction and MetHb increase	around/below 100 mg/kg bw/d by ca. 10 % MetHb increased to ca. 5 %, Heinz bodies present → Reduction in functional Hb by < 20 %	B.6.3.2/02, 1 200 ppm 2-year rat study B.6.5/03, 1 500 ppm 1-year dog study B.6.3.2/10, 1 500 ppm
(5) Haemoglobinuria that is not limited to the first three days of treatment in the repeated dose study in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥ 10 %)	Discoloured (brown or purple/black stained) urine after 3-10 doses of 1 000 mg/kg bw/d in the rat PNDT study B.6.6.2/05; Hb not measured, kidney not examined histopathologically Not present/reported in another rat PNDT study at this dose (B.6.6.2/02)	Rat PNDT studies B.6.6.2/02, /05
(6) Haemosiderinuria supported by relevant histopathological findings in the kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥ 10 %)		
(7) Multifocal or diffuse fibrosis in the spleen, liver or kidney	None	–
(8) Tubular nephrosis	None	–
(9) Marked increase of haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥ 10 %) in a 28-day study	None in the mouse; 28-day studies in other species not available A possibly "marked" increase in haemosiderosis in the liver in a 90-day dog study, Hb reduction by ca. 10 %	28-day mouse study B.6.3.1/01 90-day rat study B.6.3.2/02 90-day dog study B.6.3.2/09
(10) Significant increase in haemosiderosis in the spleen, liver or kidney in combination with microscopic effects like necrosis, fibrosis or cirrhosis	None Haemosiderosis increased, but not found in association with necrosis, fibrosis or cirrhosis up to the guidance values (single hepatocyte necrosis in association with haemosiderosis at ca. 700 mg/kg bw/d in a 90-day mouse study)	90-day rat studies B.6.3.2/02, /04 90-day mouse study 6.3.2/05 90-day dog study B.6.3.2/09 1-year dog study B.6.3.2/10 2-year rat studies B.6.5/02, /03 Two-generation rat studies B.6.6.1/01, /02

Criterion (9), i.e. "marked increase of haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥ 10 %) in a 28-day study", can be adequately assessed only for the mouse; 28-day studies are not available for rats and dogs. The rationale for specifying "in a 28-day study" is not provided in the CLP guidance nor in Muller *et al.* (2006). However, as haemosiderin deposits build up over time, a marked increase in haemosiderosis after only 4 weeks of exposure is more concerning than after 13 weeks. As to 90-day studies, an increase in haemosiderin deposition that may be considered "marked" was observed in the dog study B.6.3.2/09 at ca. 60 mg/kg bw/d (see 'Supplemental information'). Haemoglobin reduction at this dose was about 10 % (there is some uncertainty due to fluctuation and low number of animals).

The table above shows that none of the individual criteria is fulfilled. This was also the DS's conclusion. Still, the DS argued that the CLP guidance also states that in the case where

multiple less severe effects with regenerative capacity were observed, the classification should apply as, according to the CLP regulation (Annex I, 3.9.1.4), "*Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs.*" However, RAC notes that the aforementioned guidance exemplifies this with criteria (9) and (10), neither of which is met here.

Thus, while acknowledging the clear haematotoxic potential of the substance, RAC does not find the effects below the guidance values sufficiently adverse to meet the criteria for classification as outlined in the CLP guidance.

Thyroid

The thyroid-related effects below the guidance values in studies with desmedipham are summarised in Table 28 of the CLH report.

Significantly increased incidence of thyroid follicular cell hypertrophy or hyperplasia below the guidance value for classification in Category 2 was observed in several rat and dog studies (B.6.3.2/02, /04, /09, /10). The severity after 90 days was minimal to slight/mild (B.6.3.2/02, /09, /10) or minimal to moderate (B.6.3.2/04). The dog studies (B.6.3.2/09, /10) also reported an approx. 1.5-fold increase in thyroid weight.

Thyroid hormone levels were measured in the 90-day rat study (B.6.3.2/03), in the 2-year rat study (B.6.5/03) and in the 1-year dog study (B.6.3.2/10) (and in another dog study that used very low doses, B.6.3.2/08).

The 90-day rat study (B.6.3.2/03) reported a relatively marked T4 reduction (by 30/69 % in males/females (m/f)) at 300 ppm (26/27 mg/kg bw/d in m/f) after 3 months that was only partly reversible within further 4 weeks. There was no effect on thyroid hormone levels after the first 4 weeks of exposure. Interestingly, there were no histopathological findings in the thyroid in this study.

The 2-year rat study (B.6.5/03) conducted in the same strain (Wistar Han) found a statistically significant T4 reduction in females of all treated groups and in high dose males. The results are presented in the table below. Notably, the effects are much less profound than in the 90-day study (B.6.3.2/03). As to histopathological findings, incidence of follicular hyperplasia was significantly increased from 300 ppm in males and at 1 500 ppm in females. There was no increase in thyroid tumours in this study.

T4 levels (nmol/L) in the 2-year rat study, B.6.5/03				
Dose (ppm)	**0**	**60**	**300**	**1 500**
Dose (mg/kg bw/d) m/f	0	3.2/3.9	16/20	80/101
Males, after 12 months	60.3	54.1	56.3	44.2* (-27 %)
Males, after 24 months	27.1	26.0	23.5	21.5* (-21 %)
Females, after 12 months	36.3	34.3	29.2* (-20 %)	24.7* (-32 %)
Females, after 24 months	33.9	29.1* (-14 %)	20.3* (-40 %)	12.9* (-62 %)

* Statistically significant difference from control, $p \leq 0.05$

The dog study (B.6.3.2/10) reported a statistically significant T4 reduction by 39 % in females after 1 year at a dose of 57 mg/kg bw/d.

The overall picture of effects below the guidance values for classification is a clear increase in follicular cell hypertrophy/hyperplasia in both the rat and the dog, leading to T4 reduction after several months of exposure. There is however no increase in thyroid tumours in the rat. Interestingly, the rat thyroid seems to be able to compensate for some time (at least 1 month) but eventually T4 levels start to differ from controls and the difference deepens with time.

The CLH report describes a 28-day *in vivo* mechanistic study in male rats (B.6.8.2/01) and an *in vitro* study with dog hepatocytes (B.6.8.2/02) indicating that desmedipham increases UDP glucuronosyltransferase activity in the liver. This mechanism is not considered relevant for humans according to the CLP guidance. However, some alternative modes of action (e.g., interference with thyroid hormone synthesis) have not been investigated.

Although the thyroid-related effects observed in studies with desmedipham are not negligible and human relevance has not been completely excluded, RAC is of the view that the findings below the (extrapolated) guidance values still do not reach the degree of adversity warranting a STOT RE classification.

Acetylcholinesterase activity

The molecule of desmedipham contains carbamate groups. Measurement of AChE activity was included in several rat and dog studies.

According to the WHO/JMPR (1999) guidance on cholinesterase inhibition, inhibition of brain AChE activity and clinical signs are the primary endpoints of concern in toxicological studies on compounds that inhibit AChE. Erythrocyte AChE inhibition can be used as a surrogate for brain AChE inhibition when data on the brain enzyme are not available, but can also be used in the presence of brain AChE data as a surrogate for AChE inhibition in peripheral tissues. The WHO/JMPR guidance recommends that statistically significant inhibition of brain or erythrocyte AChE by ≥ 20 % is considered adverse. It also advises that statistically significant inhibition of less than 20 % or statistically insignificant inhibition above 20 % deserve further analysis of the data and might be adverse in certain cases (depending on the slope of the dose-response curve, assay variability and correlation with clinical signs).

Clinical signs of neurotoxicity (occasional signs of decreased activity, tremors, ataxia, spasms and episodes of lateral or ventral recumbency) were seen in the 1-year dog study (B.6.3.2/10) mainly at the top dose of 170 mg/kg bw/d, but 1 animal per group (of 8-12 animals) were also affected at the mid- and low dose (ca. 55 and 10 mg/kg bw/d, respectively). No effect on brain or erythrocyte AChE activity was observed in this study. Because of the low incidence of clinical signs below the guidance value and lack of effects on AChE, this study is not considered to support classification for neurotoxicity.

No clinical signs of neurotoxicity were observed in rats. Statistically significant inhibition of brain AChE activity by more than 20 % (up to 22 %) was reported below the guidance value in two 90-day rat studies (B.6.3.2/02, 04) but the effect was limited to one sex in each study (females in B.6.3.2/02, males in B.6.3.2/04) and there was no clear dose-response relationship (see Tables 33 and 35 in the CLH report). No brain AChE reduction was observed at any time point in the 2-year rat study B.6.5/01,02 up to the top dose of 1 200 ppm (75/97 mg/kg bw/d in m/f). AChE activity in erythrocytes was not affected in any of the three studies. In the absence of clinical signs or a consistent and dose-related effect on brain AChE in the rat studies, the data are not considered to justify classification.

Conclusion on classification

RAC agrees with the DS that the effects on the thyroid and nervous system observed in studies with desmedipham do not justify classification. Contrary to the DS's proposal, RAC does not consider the haematologic effects to meet the STOT RE criteria either. Thus, RAC is of the opinion that **no classification is warranted for STOT RE.**

Supplemental information - In depth analyses by RAC

Time dependence of haematological effects

The tables below show that the severity of haemoglobin reduction does not depend on exposure duration from 3 months to 2 years in the rat. The same applies to MetHb increase from 10 days to 2 years. The effective doses did not decrease with increasing study duration.

Haemoglobin reduction (compared to control) in 2-year rat study, B.6.5/03

	Dose	3 months	6 months	12 months	18 months	24 months
Males	60 ppm (3.2 mg/kg bw/d)	nss	nss	nss	nss	-8 %
	300 ppm (16 mg/kg bw/d)	-4 %	-3 %	-6 %	nss	nss
	1500 ppm (80 mg/kg bw/d)	-11 %	-11 %	-12 %	-10 %	-5 %
Females	60 ppm (3.9 mg/kg bw/d)	nss	nss	nss	nss	nss
	300 ppm (20 mg/kg bw/d)	nss	-5 %	nss	nss	-5 %
	1 500 ppm (100 mg/kg bw/d)	-9 %	-11 %	-10 %	-13 %	-9 %

nss = no statistically significant difference

Methaemoglobin (% of haemoglobin) in 2-year rat study, B.6.5/03

	Dose	3 months	6 months	12 months	18 months	24 months
Males	0 ppm	0.8	0.6	0.7	1.0	1.1
	60 ppm (3.2 mg/kg bw/d)	1.2*	0.8	0.9	1.2	1.5
	300 ppm (16 mg/kg bw/d)	2.4*	2.0*	1.8*	2.0*	2.3*
	1 500 ppm (80 mg/kg bw/d)	6.4*	6.2*	4.9*	6.0*	4.6*
Females	0 ppm	0.7	0.5	0.8	0.9	1.3
	60 ppm (3.9 mg/kg bw/d)	0.9	0.8*	1.1*	1.1	1.1
	300 ppm	1.8*	1.5*	1.6*	1.8*	1.8

	(20 mg/kg bw/d)					
	1 500 ppm (100 mg/kg bw/d)	4.7*	4.2*	3.7*	4.4*	4.4*

* statistically significant difference from control, p < 0.05

Haemoglobin reduction and methaemoglobin after 3 months in rat study, B.6.3.2/02				
	Haemoglobin reduction (compared to control)		**Methaemoglobin (% of haemoglobin)**	
	Males	Females	Males	Females
0 ppm	n.a.	n.a.	1.0	1.1
300 ppm (24/27 mg/kg bw/d m/f)	-5 %	-4 %	2.7*	2.1*
1 200 ppm (97/109 mg/kg bw/d m/f)	-9 %	-11 %	5.3*	4.0*
4 800 ppm (415/378 mg/kg bw/d m/f)	-15 %	-7 %	11.2*	8.5*

* statistically significant difference from control, p < 0.05

Methaemoglobin (% of haemoglobin) after 10 doses in rat PNDT study, B.6.6.2/03	
Control	1.3
10 mg/kg bw/d	1.6
100 mg/kg bw/d	3.7*
500 mg/kg bw/d	9.3*

* statistically significant difference from control, p < 0.05

Haemosiderosis in 28-day and 90-day studies

A 28-day study is available only for the mouse (B.6.3.1/01). Haemosiderosis of the spleen was observed in 1 male at 416 mg/kg bw/d (1 600 ppm). No haemosiderosis was observed at a similar dose (2 300 ppm) in a 90-day mouse study (B.6.3.2/05).

Increased pigmentation in the liver and spleen was reported from 1 200 ppm (ca. 100 mg/kg bw/d) in 90-day rat study (B.6.3.2/02). Severity scores are not available in the RAR. Another 90-rat study (B.6.3.2/04) reported an increase in haemosiderosis of the spleen and pigmentation of renal tubules mainly at 4 000 ppm (ca. 300 mg/kg bw/d).

Pigmentation in the liver and kidney in rat 90-day study, B.6.3.2/02				
	Dose	**No. of animals**	**Liver: pigmentation**	**Kidneys: pigmentation**
Males	0 ppm	10	0	0
	300 ppm (24 mg/kg bw/d)	10	0	0
	1 200 ppm	10	4	2

	(97 mg/kg bw/d)			
	4 800 ppm (415 mg/kg bw/d)	10	9	8
Females	0 ppm	10	0	0
	300 ppm (27 mg/kg bw/d)	10	0	0
	1 200 ppm (109 mg/kg bw/d)	10	1	0
	4 800 ppm (378 mg/kg bw/d)	10	4	0

A 90-day study in the dog (B.6.3.2/09) reported increased incidence of haemosiderin deposition in macrophages and Kupffer cells at ca. 60 mg/kg bw/d. This increase can be considered "marked". The concurrent Hb reduction was about 10 % (this value takes into account both sexes and fluctuations as shown in the table below). Study B.6.3.2/10 showed increased MetHb levels to ca. 3 %/4 % (m/f) at this dose.

Haematosiderin deposition in the liver in dog 90-day study B.6.3.2/09

	Dose	**No. of animals**	**Liver: haemosiderin in macrophages and Kupffer cells**
Males	0 ppm	4	2 (1 ±, 1 +)
	100 ppm (3.7 mg/kg bw/d)	4	1 (1 ++)
	500 ppm (19 mg/kg bw/d)	4	1 (1 +)
	1 500 ppm (57 mg/kg bw/d)	4	4 (3 +, 1 ++)
Females	0 ppm	4	2 (1 ±, 1 ++)
	100 ppm (4.1 mg/kg bw/d)	4	1 (1 ±)
	500 ppm (21 mg/kg bw/d)	4	3 (1 ±, 2 +)
	1 500 ppm (62 mg/kg bw/d)	4	4 (1 +, 1 ++, 2 +++)

Reduction in Hb levels (compared to control) at 1 500 pm in dog studies B.6.3.2/09, /10

	B.6.3.2/09		**B.6.3.2/10**		
	Week 6	**Week 12**	**Week 13**	**Week 27**	**Week 52**
Males	-8 %	-1 %	-8 %	-15 %*	-13 %
Females	-4 %	-15 %*	-9 %	-17 %*	-15 %

* statistically significant difference from control, $p < 0.05$

10.13 Aspiration hazard

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

A brief summary of relevant studies on degradation, listed in the Renewal Assessment Report (RAR), is reported below. Only the information considered adequate, reliable and relevant for the classification proposal of desmedipham has been included.

Table 40: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready biodegradability - desmedipham			
OECD TG 301 D: Ready Biodegradability: Closed bottle test (1981) Desmedipham technical (chemical purity unknown) GLP compliant	**21 %** of desmedipham was **degraded** during **28 days**.	The substance is **not readily biodegradable**.	1991 RAR B.8.2.2.1/03 M-147009-01-1 **Key study**
Hydrolysis			
Hydrolysis – desmedipham			
OECD TG 111: Hydrolysis as a function of pH (2004) * US EPA: OPPTS 835.2110: Hydrolysis as a function of pH (1998) [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity: 2.89 MBq/mg and radiochemical purity > 99 %) GLP compliant	**At pH 4:** DT_{50}: 248 d DT_{90}: 823 d EHPC: 8.1 % **At pH 5:** DT_{50}: 39 d DT_{90}: 129 d EHPC: 40.7 % **At pH 7:** DT_{50}: 12 h DT_{90}: 39 h EHPC: 98.6 % **At pH 9:** DT_{50}: 7 min DT_{90}: 22 min EHPC: 95.6 %	The study was conducted at 25 °C. The results show hydrolysis being pH depended and very rapid in alkaline conditions at 25 °C.	2003 RAR B.8.2.1.1/04 M-215909-01-1
OECD TG 111: Hydrolysis as a function of pH (2004) * US EPA: OPPTS 835.2110: Hydrolysis as a function of pH (1998)	**[^{14}C-phenoxy]-desmedipham:** **DT_{50} at pH 4**: 10 °C: - 20 °C: - 25 °C: 351 d **[^{14}C-aniline]-desmedipham & [^{14}C-phenoxy]-desmedipham:**	Study results show that the hydrolysis of ^{14}C-desmedipham is a relatively rapid reaction at neutral and especially alkaline conditions.	2005 RAR B.8.2.1.1/05 M-493908-02-1

Method	Results	Remarks	Reference
[aniline-UL-^{14}C]-desmedipham (specific activity: 1.78 GBq/mmol and radiochemical purity > 96.6 %) [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity: 1.96 GBq/mmol and radiochemical purity > 96.8 %) GLP compliant	**DT_{50} at pH 5:** 10 °C: - 20 °C: 107 d 25 °C: 58 d **DT_{50} at pH 7:** 10 °C: 7.3 d 20 °C: 26.5 h 25 °C: 13.3 h **DT_{50} at pH 9:** 10 °C: 4.4 h 20 °C: 1.0 h 25 °C: 0.6 h		
OECD TG 111: Hydrolysis as a function of pH (2004) [aniline-UL-^{14}C]-desmedipham (specific activity: 3.91 GBq/mmol and radiochemical purity > 97.9 %) GLP compliant	**DT_{50} at pH 4:** 20 °C: 4884 d 50 °C: 7 d 60 °C: 3 d **DT_{50} at pH 7**: 20 °C: 23 h 25 °C: 9 h 30 °C: 4 h **DT_{50} at pH 9:** 20 °C: 14 min 25 °C: 8 min 30 °C: 4 min	Study results show the hydrolytic degradation of desmedipham is strongly dependent on the pH of the solution.	2013 RAR B.8.2.1.1/06 M-460099-01-1
OECD TG 111: Hydrolysis as a function of pH (2004) [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity: 3.91 GBq/mmol and unknown radiochemical purity) GLP compliant	**At pH 4:** DT_{50}: 567 d DT_{90}: 1885 d **At pH 4.5:** DT_{50}: 281 d DT_{90}: 935 d **At pH 5:** DT_{50}: 82 d DT_{90}: 273 d **At pH 5.5:** DT_{50}: 29 d DT_{90}: 95 d **At pH 6:** DT_{50}: 10 d DT_{90}: 34 d **At pH 6.5:** DT_{50}: 3 d DT_{90}: 10 d **At pH 7:** DT_{50}: 23.2 h DT_{90}: 77 h **At pH 7.5:**	Study results show the hydrolytic degradation of desmedipham being strongly depended on the pH of the solution	2015 RAR B.8.2.1.1/07 M-533311-0-1

Method	Results	Remarks	Reference
	DT_{50}: 7.0 h DT_{90}: 23.3 h **At pH 8:** DT_{50}: 2.4 h DT_{90}: 23.3 h		
Hydrolysis – degradate ethyl-(3-hydroxyphenyl)-carbamate (EHPC)			
OECD TG 111: Hydrolysis as a function of pH (2004) * US EPA: OPPTS 835.2110: Hydrolysis as a function of pH (1998) [phenyl-UL-^{14}C]-EHPC (specific activity: 13.8 MBq/mg and radiochemical purity > 99 %) GLP compliant	EHPC was stable under sterile aqueous conditions at pH 4, 5, 7 and 9 for 0, 2, 4, and 120 hours at 50 °C.	EHPC was stable during the study.	2003 RAR B.8.2.1.1/08 M-227339-01-1
OECD TG 111: Hydrolysis as a function of pH (2004) [phenyl-UL-^{14}C]-EHPC (specific activity: 1.22 GBq/mmol (by MS) and radiochemical purity 98.8 %) GLP compliant	No hydrolysis was detected at pH 4, 5, 7 and 9 at a temperature of 50 °C.	EHPC is considered to be hydrolytically stable.	2005 RAR B.8.2.1.1/09 M-493918-01-1
Other convincing scientific evidence			
Inherent and enhanced ready biodegradability tests			
OECD TG 302 C: Modified Miti-test (II) (1981) Desmedipham technical (chemical purity 97.6 %) GLP compliant	45 % of desmedipham was degraded (BOD/thOD) after 28 days.		1990 RAR B.8.2.2.1/02 M-146905-01-1
Water, water-sediment and soil degradation data (including simulation studies)			
Aerobic mineralisation in surface water			
OECD TG 309: Simulation biodegradation test (2004) [aniline-UL-^{14}C]-desmedipham	**Desmedipham DT_{50}:** High conc.: 0.004 d Low conc.: 0.12 d **Degradate aniline DT_{50}:** High conc.: 75.9 d Low conc.: 34.9 d	The half-life of diphenyl urea could be estimated for high concentration test systems only. Kinetic evaluation of the data has been performed	2014 RAR B.8.2.2.2/01 & RAR B.8.2.2.2/02 M-477210-01-1 & M-496960-01-1

Method	Results	Remarks	Reference
(specific activity: 3.91 MBq/mg and radiochemical purity > 98 %) FOCUS DegKinetics Report (2006) GLP compliant	**Degradate diphenyl urea DT_{50}:** High conc.: 2.7 d	according to the final report of the work group on degradation kinetics of FOCUS, guidance document on estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration, aka FOCUS DegKinetics Report (2006).	**Key study**
Water-sediment data			
OECD TG 308: Aerobic and anaerobic transformation in aquatic sediment systems (2008) * [aniline-UL-^{14}C]-desmedipham (specific activity: 50.2 µCi/mmol and radiochemical purity > 97 %) FOCUS DegKinetics Report (2006) GLP compliant	Mean values of maximum occurrences measured: **Radioactivity in water phase:** Rhine River: 98.4 % of AR (day 0) and 1.2 % (day 105) Anwil Pond: 96.4 % of AR (day 0) and 0.7 % (day 105) **Distribution into sediment:** Rhine River: 38.3 % of AR after 1 day and 20.6 % after 105 days Anwil Pond: 36.9 % of AR after 1 day and 32.2 % after 105 days **Aniline formation in water:** Rhine River: 54.4 % of AR (day 0.25) Anwil Pond: 55.1 % of AR (day 0.25) **Diphenyl urea formation in water:** Rhine River: 4.3 % of AR (day 0.25) Anwil Pond: 4.4 % of AR (day 0.25) **Non-extracted residues (NER):** The non-extractable residues were found in the humin fraction 11.7 % and 10.7 % for the two river samples and 29.2 % and 20.9 % for the two pond samples on day 105. **Mineralisation (mainly to CO_2)**: Rhine River: 66.4 % of AR (day 105) Anwil Pond: 56.0 % of AR (day 105) **DT_{50} in total systems:** Rhine River: 0.035 d for desmedipham and 0.23 d for aniline Anwil Pond: 0.045 d for desmedipham **DT_{50} in water phase:** Rhine River: 0.024 d for desmedipham and 0.14 d for aniline Anwil Pond: 0.041 d for desmedipham	The later sampling points (after day 14) showed no loss of radioactivity which was explained to result from high losses of volatiles (mainly $^{14}CO_2$) during processing of the samples in frequent intervals. The kinetic evaluation followed in other aspects also the FOCUS DegKinetics Report (2006). AR = applied radioactivity	1994 & 1995 RAR B.8.2.2.3/05 & RAR B.8.2.2.3/06 M-147032-01-1 & M-147031-01-1
OECD TG 308: Aerobic and	Mean values of maximum occurrences measured:	Formation of major degradation products	2003

Method	Results	Remarks	Reference
anaerobic transformation in aquatic sediment systems (2002) * SETAC guideline: Procedures for assessing the environmental-fate and ecotoxicity of pesticides: Part 1: Section 8.2: Aerobic aquatic degradation (1995) US EPA: OPPTS 835.4300: Aerobic Aquatic Metabolism (2008) [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity: 78.17 µCi/mmol and radiochemical purity 99.5 > 98.4 %) [aniline-UL-^{14}C]-desmedipham (specific activity: 105.74 µCi/mg and radiochemical purity 99.5 > 99.1 %) FOCUS DegKinetics Report (2006) GLP compliant	**Radioactivity OCR pond water phase:** [^{14}C-phenoxy]-desmedipham: 96.1 % of AR (day 0) and 13.4 % of AR (day 100) [^{14}C-aniline]-desmedipham: 94.3 % of AR (day 0) and 9.7 % of AR (day 100) **Radioactivity in CMS pond water phase:** [^{14}C-phenoxy]-desmedipham: 97.0 % of AR (day 0) and 36.2 % of AR (day 100) [^{14}C-aniline]-desmedipham: 95.1 % of AR (day 0) and 20.8 % of AR (day 100) **Distribution into sediment after 100 days incubation:** OCR pond: 57.9 % of AR ([^{14}C-phenoxy]-desmedipham) and 17.1 % of AR ([^{14}C-aniline]-desmedipham) CMS pond: 45.1 % of AR ([^{14}C-phenoxy]-desmedipham) and 34.1 % of AR ([^{14}C-aniline]-desmedipham) **Degradation product formation in OCR pond water phase:** EHPC: 95.7 % of AR (day 1) Aniline: 71.9 % of AR (day 21) **Degradation product formation in CMS pond water phase:** EHPC: 59.1 % of AR (day 0) Aniline: 62.5 % of AR (day 14) **Non-extracted residues (NER) in OCR pond after 100 days incubation:** [^{14}C-phenoxy]-desmedipham: 56.2 % [^{14}C-aniline]-desmedipham: 15.9 % **Non-extracted residues (NER) in CMS pond after 100 days incubation:** [^{14}C-phenoxy]-desmedipham: 28.8 % [^{14}C-aniline]-desmedipham: 30.1 % **Mineralisation to CO_2 after 100 days incubation in:** OCR pond: 43.7 % CMS pond: 14.6 % Geometric means from kinetic evaluation of the data: **Desmedipham DT_{50} and DT_{90} in**	aniline and EHPC were studied with two separate tests. The tests were carried out both in sandy loam pond (OCR pond) and in clay loam pond (CMS pond). The majority of the unextractable residue in OCR sediment was found in humin and fulvic acid fractions of the soil. The unextractable residue in the CMS sediment was found in significant quantities not only in the humin and fulvic acid fractions but also in the humic acid fraction. Mean values of mineralisation to CO_2 after 100 days incubation were calculated by dossier submitter from data presented in the RAR.	RAR B.8.2.2.3/07 & RAR B.8.2.2.3/08 M-218087-01-1 & M-228989-01-1

Method	Results	Remarks	Reference
	total systems: OCR pond: 0.13 d (DT_{50}) and 0.43 d (DT_{90}) CMS pond: 3.1 d (DT_{50}) and 12.6 d (DT_{90}) **Degradate EHPC DT_{50} in total systems:** OCR pond: 50.3 d CMS pond: 64.2 d **Degradate aniline DT_{50} in total systems:** OCR pond: 5.3 d CMS pond: 42.0 d		
OECD TG 308: Aerobic and anaerobic transformation in aquatic sediment systems (2002) * [aniline-UL-^{14}C]-desmedipham (specific activity: 105.74 µCi/mg and radiochemical purity 99.5 > 99.1 %) GLP compliant	Mean values of maximum occurrences measured: **Radioactivity in OCR pond total system:** Desmedipham: 17.3 % of AR (day 0.25), 0.0 % of AR (day 14) and 0.0 % of AR (day 100) Degradate aniline: 71.9 % of AR (day 0.25), 0.8 % of AR (day 14) and 0.8 % of AR (day 100) Degradate phenol: 0.9 % of AR (day 0.25), 2.2 % of AR (day 14) and 0.2 % of AR (day 100) **Radioactivity in CMS pond total system:** Desmedipham: 93.7 % of AR (day 0), 4.9 % of AR (day 14) and 0.9 % of AR (day 100) Degradate aniline: 0.6 % of AR (day 0), 62.6 % of AR (day 14) and 15.5 % of AR (day 100) Degradate phenol: 0.0 % of AR (day 0), 3.4 % of AR (day 14) and 1.2 % of AR (day 100) **Desmedipham DT_{50} in total systems:** OCR pond: 0.1 d CMS pond: 2.6 d **Degradate aniline DT_{50} in total systems:** OCR pond: 5.8 d CMS pond: 47.1 d **Degradate phenol DT_{50} in total systems:** OCR pond: 0.3 d CMS pond: 4.3 d	The kinetic evaluation of the degradation of desmedipham to phenol was performed with ACSL Optimize Software package.	2003 RAR B.8.2.2.3/09 M-221610-01-1
OECD TG 308: Aerobic and anaerobic	Mean values of maximum occurrences measured:	For statistical analysis, a few different kinetic models were fitted to the	2003 RAR B.8.2.2.3/10

Method	Results	Remarks	Reference
transformation in aquatic sediment systems (2002) [aniline-UL-^{14}C]-desmedipham (specific activity: 31.20 mCi/mmol and unknown radiochemical purity) FOCUS DegKinetics Report (2006) non GLP	**Radioactivity in Turkey Creek total system:** Desmedipham: 92.2 % of AR (day 0), 49.6 % of AR (day 1) and 4.2 % of AR (day 14) Degradate aniline: 0.5 % of AR (day 0), 16.2 % of AR (day 1) and 0.9 % of AR (day 14) **Radioactivity in Choptank River total system:** Desmedipham: 73.8 % of AR (day 0), 16.8 % of AR (day 1) and 0.1 % of AR (day 63) Degradate aniline: 3.4 % of AR (day 0), 11.5 % of AR (day 1) and 0.2 % of AR (day 63) **Non-extracted residues (NER) after 100 days incubation in:** Turkey Creek: 45.8 % Choptank River: 33.7 % **Desmedipham DT_{50} in total system:** Turkey Creek: 1.3 d (FOMC) Choptank River: 0.37 d (FOMC) **Desmedipham DT_{90} in total system:** Turkey Creek: 6.7 d (FOMC) Choptank River: 2.8 d (FOMC) **Desmedipham DT_{50} in water phase:** Turkey Creek: 1.1 d (FOMC) Choptank River: 0.51 d (FOMC) **Mineralisation to CO_2 after 100 days incubation in:** OCR pond: 32.5 % CMS pond: 31.8 %	data in order to find the one that can predict best the experimental results. Abbreviations of the models implement using the software KinGUI 2.1 are SFO (Single First-Order), FOMC (First Order Multi-Compartment), HS (Hockey Stick) and DFOP (Double First-Order in Parallel).	M-493999-01-1
OECD TG 308: Aerobic and anaerobic transformation in aquatic sediment systems (2002) * SETAC guideline: Procedures for assessing the environmental-fate and ecotoxicity of pesticides: Part 1: Section 8.2: Aerobic aquatic degradation (1995) [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity:	Mean values of maximum occurrences measured (with TLC & HPLC): **Radioactivity in OVP total system:** Desmedipham: 109.5 % of AR (day 0.2), 1.8 % of AR (day 0.21) and not detected (day 49) Degradate EHPC: 1.3 % of AR (day 0.2), 93.2 % of AR (day 0.21) and 0.8 % of AR (day 49) **Radioactivity in SW total system:** Desmedipham: 99.9 % of AR (day 0.2), 2.7 % of AR (day 0.21) and not detected (day 49) Degradate EHPC: 2.6 % of AR (day 0.2), 95.7 % of AR (day 0.21) and 0.2 % of AR (day 49) **Non-extracted residues (NER) after**	Data are available for both the water and sediment compartments as well as the total system. However, two analytical techniques have been used to measure residues with no clear indication as to which is the preferred method (both have given good results and recoveries). For the kinetic analysis of half-lives, both sets were analysed and the **worst case kinetics** were reported. For radioactivity, the mean values of the two analytical techniques are presented.	2003 RAR B.8.2.2.3/11 M-494009-01-1

Method	Results	Remarks	Reference
3.52 MBq/mg and radiochemical purity > 98.6 %) FOCUS DegKinetics Report (2006) GLP compliant	**100 days incubation in:** OVP: 70.8 % SW: 68.6 % **Desmedipham DT_{50} in total system:** OVP: 0.00022 d (SFO_{TLC}) SW: 0.055 d (SFO_{HPLC}) **Desmedipham DT_{50} in water phase:** OVP: 0.034 d (SFO_{HPLC}) SW: 0.039 (SFO_{HPLC}) **Degradate EHPC DT_{50} in total system:** OVP: 9.6 d (SFO_{TLC}) SW: 6.4 d (SFO_{TLC}) **Degradate EHPC DT_{50} in water phase (worst case kinetic analysis):** OVP: 6.3 d (SFO_{HPLC}) SW: 4.8 d (SFO_{TLC}) **Mineralisation to CO_2 after 100 days incubation in:** OVP: 36.2 % SW: 29.1 %		
OECD TG 308: Aerobic and anaerobic transformation in aquatic sediment systems (2002) * [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity: 1.96 GBq/mmol and radiochemical purity 99.5 %) FOCUS DegKinetics Report (2006) GLP compliant	Mean values of maximum occurrences measured: **Radioactivity in Illingen pond total system:** Desmedipham: 16.8 % of AR (day 0), 0.0 % of AR (day 1) and 0.0 % of AR (day 121) Degradate EHPC: 82.7 % of AR (day 0), 91.3 % of AR (day 1) and 7.6 % of AR (day 121) **Radioactivity in Dentelbach creek total system:** Desmedipham: 22.2 % of AR (day 0), 0.0 % of AR (day 1) and 0.0 % of AR (day 121) Degradate EHPC: 78.0 % of AR (day 0), 96.3 % of AR (day 1) and 7.7 % of AR (day 121) **Non-extracted residues (NER) after 100 days incubation in:** Illingen pond: 62.8 % Choptank River: 55.1 % **Desmedipham DT_{50} in total system:** Illigen pond: 0.052 d (SFO) Dentelbach creek: 0.050 d (SFO) **Desmedipham DT_{50} in water phase:** Illigen pond: 0.052 d (SFO) Dentelbach creek: 0.050 d (SFO)	The available data for EHPC in the sediment from both the Illingen and Dentelbach creek was just four time points, which according to (FOCUS, 2006) is insufficient to derive a reliable DT_{50}. However, this was attempted and the reliability of the DT_{50} derived judged on the quality of fit and statistics. Evaluation of kinetic fit at level II was tested but failed for both systems (Illingen pond: χ^2 -error fails for both compartments; the sediment fit was very poor and the t-test failed. Dentelbach Creek: χ^2 -error failed for the sediment compartment; the sediment fit was very poor and the t-test failed).	2003 RAR B.8.2.2.3/12 M-494005-01-1

Method	Results	Remarks	Reference
	Degradate EHPC DT_{50} in total system: Illigen pond: 10.1 d (SFO) Dentelbach creek: 23.4 d (SFO) **Degradate EHPC DT_{50} in water phase:** Illigen pond: 7.0 d (SFO) Dentelbach creek: 17.5 d (SFO) **Degradate EHPC DT_{50} in sediment:** Illigen pond: 48.4 d (SFO) Dentelbach creek: 54.7 d (SFO) **Mineralisation to CO_2 after 100 days incubation in:** Illingen pond: 20.4 % CMS pond: 28.8 %		
Soil degradation data			
Aerobic degradation in soil – desmedipham			
FOCUS DegKinetics Report (2006) Non GLP	Pathway scheme for the degradation of desmedipham in soil.		2014 RAR B.8.1.1.1.1/01 M-496955-01-1
OECD TG 307: Aerobic and anaerobic transformation in soil (2002) * [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity: 2.28 MBq/mg and radiochemical purity > 96 %) FOCUS DegKinetics Report (2006) GLP compliant	**Non-normalized SFO DT_{50} value** of 8.8 days in German standard soil 2.3 was obtained. For **EHPC**, no acceptable fit could be obtained.	The study was basically performed in line with the OECD test guideline 307 (2002), except no replicates were included in the study.	1991 RAR B.8.1.1.1.1/06 M-146918-01-1
OECD TG 307: Aerobic and anaerobic transformation in soil (2002) * [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity: 220.2 µCi/mg and radiochemical purity 99.42 %) [aniline-UL-^{14}C]-desmedipham	**Non-normalized best fit DT_{50} values** in: Speyer 2.2: 28.2 d (SFO) sandy loam: 25.1 d (SFO) acidic clay loam: 33.1 d (SFO) alkaline clay loam: 8.0 d (FOMC)	The total radioactive recovery was close to 100 % which fulfils the quality criteria in OECD test guideline.	1991 RAR B.8.1.1.1.1/07 M-147006-01-1

Method	Results	Remarks	Reference
(specific activity: 221.4 µCi/mg and radiochemical purity 99.36 %) FOCUS DegKinetics Report (2006) GLP compliant			
OECD TG 307: Aerobic and anaerobic transformation in soil (2002) * [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity: 85.95 µCi/mg and radiochemical purity > 95 %) FOCUS DegKinetics Report (2006) GLP compliant	**Non-normalized DFOP DT_{50}** for trigger evaluation: 3.7 d **Pseudo SFO DT_{50}** for modelling purposes: 28.2 d **EHPC non-normalized SFO DT_{50}** for trigger and modelling evaluation: 1.4 d	The study was conducted using German standard soil 2.1.	1993 RAR B.8.1.1.1.1/08 M-146937-01-1
Aerobic degradation in soil –degradate EHPC			
OECD TG 307: Aerobic and anaerobic transformation in soil (2002) * [phenyl-UL-^{14}C]-EHPC (specific activity: 13.8 MBq/mg and radiochemical purity 100 %) FOCUS DegKinetics Report (2006) GLP compliant	Best fit **DT_{50} values** in: sandy loam 1: 0.07 d (FOMC) sandy loam 2: 0.26 d (SFO) clay loam: 0.39 d (SFO)	The mean total radioactivity recovery from all soil types was **96 %**. All individual mass balances were within the range **90.9** to **104.5 %** of applied radioactivity, except for 1 sample (recovery of **88.0 %**) where some loss of sample occurred due to a centrifuge tube breaking during extraction of the soil.	2003 RAR B.8.1.1.1.2/02 M-217961-01-1
OECD TG 307: Aerobic and anaerobic transformation in soil (2002) * [phenyl-UL-^{14}C]-EHPC (specific activity: 618 MBq/mmol and radiochemical purity > 98 %)	Best fit **DT_{50} values** in: Speyer 2.1: 8.7 d (SFO) Speyer 2.2: 3.5 d (SFO) Speyer 2.3: 1.0 d (FOMC) Best fit **DT_{90} values** in: Speyer 2.1: 11.6 d Speyer 2.2: 7.9 d Speyer 2.3: 10.1 d	For Speyer soils 2.1., 2.2 and 2.3., the **mass balance** varied from 82 to 103 % of AR (average 93% of AR) from 88 to 101 % of AR (average 93 % of AR) and from 76 to 99 % of AR (average 91 % of AR), respectively. The amount of **carbon dioxide** steadily increased	1997 RAR B.8.1.1.1.2/03 M-493970-01-1

Method	Results	Remarks	Reference
FOCUS DegKinetics Report (2006) GLP compliant		up to 28, 25 and 17 % of AR at day 61 in soils Speyer 2.1, Speyer 2.2 and Speyer 2.3, respectively. The amount of **non-extractables (NER)** also steadily increased up to about 83 % of AR (day 14), up to about 79 % of AR (day 7) and up to about 80 % of AR (day 2) in soils Speyer 2.1, 2.2 and 2.3, respectively.	
Aerobic degradation in soil – degradate aniline			
Guideline not specified Non GLP	Degradate aniline **DT_{50} values** transformed from rate coefficient for disappearance: model 1: 1.35 d model 2: 0.018 d	Two mathematical models were evaluated for describing the radioactivity disappearance data: **model 1** represents an overall rate coefficient for the disappearance pattern of aniline. In **model 2** a larger degradation rate coefficient was predicted.	1987 RAR B.8.1.1.1.2/05 M-235824-01-1
Aerobic degradation in soil – degradate phenyl urethane			
OECD TG 307: Aerobic and anaerobic transformation in soil (2002) Phenyl urethane technical (chemical purity 99.9 %) GLP compliant	Degradate phenyl urethane **DT_{50} values** measured from different soils: Laacher Hof AXXA: 4.5 h Hoefchen am Hohenseh: 3.8 h Dollendorf: 1.3 h Laacher Hof Wurmwiese: 4.3 h **DT_{90} values** measured from different soils: Laacher Hof AXXA: 15.0 h Hoefchen am Hohenseh: 12.6 h Dollendorf: 4.4 h Laacher Hof Wurmwiese: 22.5 h	The study was performed in line with the OECD test guideline 307 (2002), except that open systems were used and therefore volatiles could not be collected. The rate of degradation of unlabelled phenyl urethane was studied in four soils under aerobic conditions in the dark in the laboratory for up to 48 hours at 20 °C. According to RAR, the results indicate that phenyl urethane will be very well degraded under aerobic conditions in the environment.	2014 RAR B.8.1.1.1.2/06 & RAR B.8.1.1.1.2/07 M-500531-01-1 & M-501958-01-1
Photochemical degradation			
Photodegradation in water			
US EPA: Pesticide Assessment Guidelines Subdivision N: Photodegradation studies in water (1982) Desmedipham technical (chemical purity ≥ 97 %)	Desmedipham **was not degraded** after 15 days exposure.	The photodegradation of desmedipham was insignificant.	1992 RAR B.8.2.1.2/02 M-146942-01-1

Method	Results	Remarks	Reference
GLP compliant			
OECD TG 316: Phototransformation of Chemicals in Water – Direct Photolysis (2008) * Desmedipham technical (chemical purity unknown) GLP compliant	**No degradation of desmedipham** occurred in irradiated samples after 18 days of exposure.	Photodegradation of desmedipham at pH 4 is shown to be insignificant.	1992 RAR B.8.2.1.2/03 M-146920-01-1
EPA Provisional Chemical Fate Guidelines 795.70 (1988) * OECD Draft TG: Photo transformation of chemicals in water, part A (1990) * Desmedipham technical (chemical purity unknown) GLP compliant	**Amount** of **desmedipham** in **irratiated samples** after **144 hours** in: synthetic water: 36.9 % distilled water: 98.9 % after **240 hours** in: synthetic water: 21.4 % distilled water: 100.4 % **DT_{50}** in synthetic natural water: 106 h	According to RAR, the results of the photolysis experiments with desmedipham indicate that the substance which is almost not photodegradable in distilled water within six days could undergo photodegradation in natural waters.	1994 RAR B.8.2.1.2/04 M-146938-01-1
OECD TG 316: Photo transformation of Chemicals in Water – Direct Photolysis (2008) * OECD Draft TG: Photo transformation of Chemicals in Water - Direct and Indirect Photolysis (2000) [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity: 1.07 GBq/mmol and radiochemical purity > 95 %) [aniline-UL-^{14}C]-desmedipham (specific activity: 1.15 GBq/mmol and radiochemical purity ≥ 95 %) GLP compliant	At pH 5 **no direct photo transformation reactions** occur after **29 days** of continuous exposure.	According to RAR, determination of photolysis at pH 7 and 9 was severely hampered by hydrolysis. Therefore, no curve fitting and kinetic evaluations were performed.	2004 RAR B.8.2.1.2/06 M-493941-01-1
OECD TG 316:	Desmedipham **DT_{50} values** in natural	For desmedipham, there	2004

Method	Results	Remarks	Reference
Phototransformation of Chemicals in Water – Direct Photolysis (2008) * JMAF: 13 Seisan No. 3986. Oct 10. 2001. 2-6-2. (amended June 26. 2001) [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity: 3.28 GBq/mmol and radiochemical purity > 99 %) GLP compliant	water at 25 °C in irradiated: 0.17 h dark: 0.27 h Desmedipham **DT_{90} values** in natural water at 25 °C in irradiated: 0.57 h dark: 0.89 h Degradate EHPC **DT_{50} values** in irratiated natural water at 25 °C: 1.24 d	was no significant difference between the data for the irradiated and non-irradiated experiments which, according to RAR, reflects **hydrolytic** rather than photolytic degradation. The primary hydrolysis degradate, EHPC, was degraded due to photolysis to form numerous photolysis products.	RAR B.8.2.1.3/01 M-236995-01-1
Photodegradation in water and quantum yield			
OECD TG 316: Phototransformation of Chemicals in Water – Direct Photolysis (2008) * OECD Draft TG: Photo transformation of Chemicals in Water - Direct and Indirect Photolysis (2000) [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity: 1.96 GBq/mmol and radiochemical purity ≥ 99.5 %) [aniline-UL-^{14}C]-desmedipham (specific activity: 1.78 GBq/mmol and radiochemical purity ≥ 99.6 %) GLP compliant	The calculated **half-lives** of desmedipham at 52° North were between **10 days** and **2.8 years**, depending on the solar irradiance intensity at the respective months. A mean **quantum yield** of 1.46×10^{-3} molecules degraded per photon in distilled water was calculated.	According to RAR, direct photolysis is considered to be a process of little importance for decomposition of desmedipham in surface water.	2002 RAR B.8.2.1.2/07 M-493924-01-1
Photodegradation in soil			
OECD guidelines for the testing of chemicals: proposal for a new guideline: Phototransformation of Chemicals on Soil Surfaces (2002) * SETAC guideline:	[phenoxy-ring-UL-^{14}C]-desmedipham **DT_{50} values** in **test system**: dark: 78.3 d (SFO) irradiated: 37.9 d (SFO) [phenoxy-ring-UL-^{14}C]-desmedipham **DT_{50} values** according to **soil photolysis** in**:** **laboratory condition**s: 107 d (DFOP)	The temperature range was larger (20 ± 5 °C) than required 20 ± 2 °C. The effect of photolysis could not be avaluated for ^{14}C-aniline labelled desmedipham because the data set contains only three data points for ^{14}C-aniline	2003 RAR B.8.1.1.3.1/04 M-493965-01-1

Method	Results	Remarks	Reference
Procedures for assessing the environmental-fate and ecotoxicity of pesticides (1995) [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity: 1.96 GBq/mmol and radiochemical purity ≥ 99.5 %) [aniline-UL-^{14}C]-desmedipham (specific activity: 1.78 GBq/mmol and radiochemical purity ≥ 99.6 %) GLP compliant	**converted to summer sunlight (at 30° to 50°N):** 286 d (DFOP)	labelled desmedipham. No aniline was detected in the study.	
OECD guidelines for the testing of chemicals: Proposal for a new guideline: Phototransformation of Chemicals on Soil Surfaces (2002) [phenoxy-ring-UL-^{14}C]-desmedipham (specific activity: 1.07 GBq/mmol and radiochemical purity > 95 %) [aniline-UL-^{14}C]-desmedipham (specific activity: 1.15 GBq/mmol and radiochemical purity ≥ 95 %) GLP compliant	[phenoxy-ring-UL-^{14}C]-desmedipham **DT_{50} values** according to **soil photolysis** in**:** **laboratory condition**s: 117.9 d (SFO) **converted to summer sunlight (at 30° to 50°N):** 439.7 d (DFOP) [aniline-UL-^{14}C]-desmedipham **DT_{50} values** according to **soil photolysis** in**:** **laboratory condition**s: 151.8 d (SFO) **converted to summer sunlight (at 30° to 50°N):** 566.1 d (DFOP)	The **geometric mean DT_{50}** value of **the two labels** is 499 days at summer sunlight at 30° to 50°N.	2004 RAR B.8.1.1.3.1/05 M-493968-01-1
Photodegradation in air			
Atkinson method non GLP	**Half-life ($t_{½}$)**: 10.8 h	The estimation of atmospheric half-life is based on the estimation of desmediphams OH-reactivity according to the method of Atkinson.	1992 RAR B.8.3.1/01 M-146921-01-1

* According to the RAR, the study was conducted generally in line with the test method.

11.1.1 Ready biodegradability

A ready biodegradability study (**RAR B.8.2.2.1/03**, **1991**) was available in the RAR. The test followed OECD test guideline 301D "Closed-Bottle-Test" test guideline and was performed in lower concentration (2

mg/L) than the water solubility of desmedipham (7 mg/L). 21 % of desmedipham was degraded after 28 days. As the degradation (% of theoretical oxygen demand) of the substance is lower than the trigger value of 60 % within 28 days for respirometric methods, desmedipham is not considered readily biodegradable.

Ready biodegradability studies are among the preferred type of test data in the assessment of rapid degradability. The endpoint is presented in table (Table 40) above.

11.1.2 BOD_5/COD

No studies available.

11.1.3 Hydrolysis

Four studies on hydrolytic degradation for desmedipham and two for degradate EHPC were considered valid in the RAR. The studies followed the OECD test guideline 111 "Hydrolysis as a function of pH". The estimated half-lives ranged from 4884 days at pH 4 to 4 minutes at pH 9 at 20 °C i.e. **hydrolysis** of desmedipham **is strongly dependent on the** pH of the solution. Based on the results, the **hydrolysis** will be **rapid** in neutral and **alkaline environments** such as many natural waters. The results also show that the amounts of aniline and EHPC increased towards the end of the studies indicating that these degradates do not hydrolyse. Degradate EHPC was confirmed being **hydrolytically stable** since less than 10 % hydrolysis was detected after five days at pH 4-9 at 50 °C.

Primary degradation studies i.e. via hydrolysis combined with hazard assessment of degradation products are among the preferred type of test data in the assessment of rapid degradability. The endpoints are presented in table (Table 40) above and the studies are summarized below.

Study 1 – desmedipham

The abiotic hydrolysis of [phenoxy-ring-UL-^{14}C]-desmedipham was investigated (**RAR B.8.2.1.1/04**, **2003**) in a sterile aqueous buffer at pH 4, 5, 7 and 9 at 25 °C in the dark and characterised by HPLC. The study followed the OECD test guideline 111 (2004). Desmedipham was hydrolysed to EHPC and no other hydrolysis products were found or volatile components were formed at any pH. At pH 4 the maximum percentage of hydrolysed EHPC was 8.1 % of the applied radioactivity (AR) after 720 hours and at pH 5 40.7 % of AR after 720 hours. At pH 7 and at pH 9 the active substance was totally hydrolysed to EHPC after 72 hours and after 30 minutes, respectively. The half-lives of desmedipham at pH 4 and 5 were 248 days and 39 days, respectively. At pH 7 and 9, the half-lives of desmedipham were 12 hours and 7 minutes, respectively.

Study 2 – desmedipham

The hydrolysis rate of [aniline-UL-^{14}C]- and [phenoxy-ring-UL-^{14}C]-desmedipham was determined (**RAR B.8.2.1.1/05**, **2005**) in aqueous solution at pH 4, pH 5, pH 7 and pH 9. The study followed the OECD test guideline 111 (2004). In case of aniline-labelled desmedipham the detected hydrolysis product was exclusively aniline, in case of phenoxy-labelled desmedipham only EHPC was detected during the present hydrolysis study. At pH 9 a DT_{50} values of 0.6 hours at 25 °C, one hour at 20 °C and 4.4 hours at 10 °C were determined. At pH 7 DT_{50} values of 13.3 hours at 25 °C, 26.5 hours at 20 °C and of 7.3 days at 10 °C were determined. In acidic aqueous solution (pH 5), desmedipham is relatively stable with half-life times of about 58 days at 25 °C and of 107 days at 20 °C. These DT_{50} values represent the mean values of both test items, phenoxy- and aniline-labelled desmedipham. For phenoxy-labelled desmedipham at the test condition of pH 4 at 25 °C a DT_{50} value of 351 days was determined. At the test conditions of pH 5 at 10 °C and of pH 4 at the temperatures 10 °C and 20 °C the hydrolysis reaction was less than 5 % for the both test items.

Study 3 – desmedipham

The rate of hydrolysis of [aniline-UL-^{14}C]-desmedipham was investigated (**RAR B.8.2.1.1/06**, **2013**) at different pH values by quantifying the test item concentration after different incubation periods and at different temperatures. The main tests were performed in buffered solutions at pH 4, 7 and 9 at three different temperatures each (pH 4: 20, 50 and 60 °C; pH 7, 9: 20, 25 and 30 °C). The study followed the OECD test guideline 111 (2004). To verify the results obtained at pH 7 the test was repeated under almost equal conditions: the samples were incubated at 19 °C, 25 °C and 30 °C for 45 h, 30 h and 14 h, respectively. Screening for degradation products (aniline and EHPC) was performed by HPLC-UV and HPLC/radiodetection (EHPC in test samples were co-chromatographed with EHPC standard). Desmedipham was found to be hydrolytically stable at pH 4 and 20 °C. At pH 4: 50 °C and 60 °C; pH 7: 20 °C, 25 °C and 30 °C and pH 9: 20 °C, 25 °C and 30 °C, a significant degradation of the test item was observed. Reaction rate constants and corresponding half-lives at 20 °C were calculated to be $1.42*10^{-4}$ d^{-1} and 4884 days at pH 4, 0.030 d^{-1} and 23 hours at pH 7 and 0.051 d^{-1} and 14 minutes at pH 9, respectively. At pH 7 an unknown transformation product was found. It was rapidly transformed to aniline and therefore would be assumed to be the unstable intermediate phenyl carbamic acid which will be transformed under CO_2 elimination to aniline.

Study 4 – desmedipham

The hydrolysis of desmedipham was investigated (**RAR B.8.2.1.1/07**, **2015**) at 20 °C in sterile aqueous buffer solutions at pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 in the dark for a maximum of 30 days. The study was performed with [phenoxy-ring-UL-^{14}C]-desmedipham and a nominal test concentration of 3.23 mg/L desmedipham according to the OECD test guideline 111 (2004). The mean calculated half-lives of desmedipham ranged from 567 days at pH 4.0 to 2.4 h at pH 8.0.

Study 5 – EHPC

The hydrolysis of [phenyl-UL-^{14}C]-EHPC in aqueous buffers was evaluated (**RAR B.8.2.1.1/08**, **2003**) at pH 4, 5, 7, and 9. The study followed the OECD test guideline 111 (2004). [^{14}C]-EHPC was characterized by its HPLC retention time and the relative retention time of the compound in standard solutions. EHPC was stable under sterile aqueous conditions at pH 4, 5, 7, and 9 for 0, 2, 4, and 120 hours at 50 °C.

Study 6 – EHPC

The hydrolysis behaviour of [phenyl-UL-^{14}C]-EHPC was determined (**RAR B.8.2.1.1/09**, **2005**) in aqueous solution at pH 4, 5, 7 and 9 at a temperature of 50 °C at concentration of to 4.76 mg EHPC/L according to OECD test guideline 111 (2004). The analytical measurements were performed by HPLC with UV-detection and radioactive monitoring by LSC. No hydrolysis of EHPC was detected after 5 days at 50 °C.

11.1.4 Other convincing scientific evidence

11.1.4.1 Inherent and enhanced ready biodegradability tests

One inherent biodegradability study (**RAR B.8.2.2.1/02**, **1990**) according to OECD test guideline 302C "Modified Miti-test (II)" was available in the RAR. The test was performed in higher concentration (30 mg/L) than the water solubility of desmedipham (7 mg/L). Desmedipham was not totally soluble during the whole test period and 45 % of desmedipham was degraded (BOD/thOD) after 28 days. Inherent biodegradation studies are considered as supportive type of test data in the assessment of rapid degradability for classification purposes. The endpoint is presented in table (Table 40) above.

11.1.4.2 Water, water-sediment and soil degradation data (including simulation studies)

Aerobic mineralisation of [aniline-UL-^{14}C]-desmedipham and its degradates in surface water were investigated under defined laboratory conditions in the dark according the OECD test guideline 309 "Simulation biodegradation test". In conclusion, the **primary degradation** of desmedipham and diphenyl urea **is fast** in natural surface water systems. The calculated half-lives of desmedipham was **0.004** days in the high concentration test systems and **0.12** days in low concentration test systems. The half-lives of aniline were **75.9** days and **34.9** days for high and low concentrations, respectively, and **2.7** days for diphenyl urea high concentration test systems. Surface water simulation studies are among the preferred types of test data in the assessment of rapid degradability.

Six studies on the route and rate of degradation of [aniline-UL-^{14}C]- and [phenoxy-ring-UL-^{14}C]-labelled desmedipham in water/sediment systems under aerobic conditions were considered valid in the RAR. The studies followed the OECD test guideline 308 "Aerobic and anaerobic transformation in aquatic sediment systems". Further kinetic evaluation of the dissipation of desmedipham was performed according to FOCUS kinetics (2006). The results are based on the worst-case outcomes of kinetic analyses.

The estimated half-lives of desmedipham ranged from 0.035 to 3.1 days in total system and from 0.024 to 4 days in water phase. The estimated half-lives in total system of degradates aniline, EHPC and phenol ranged from 0.23 to 47.1 days, from 6.4 to 64.2 days and from 0.3 to 4.3 days, respectively. Based on the results, the **primary degradation** of desmedipham and the degradate phenol will be **rapid** in **natural environments**. The degradates aniline and EHPC, however, were less **degradable**.

Various studies of degradation in soil under aerobic conditions for desmedipham and its degradation products were considered valid in the RAR. The studies were performed according the OECD test guideline 307 "Aerobic and anaerobic transformation in soil" (with one exception) followed by further kinetic evaluation according to FOCUS kinetics (2006). The results are based on the worst-case outcomes of kinetic analyses i.e. the models predicting the longest half-lives.

The estimated half-lives of desmedipham in soil ranged from 3.7 to 127.2 days. The estimated half-lives of degradates aniline, EHPC and phenyl urethane ranged from 26 minutes to 1.35 days, from 100 minutes to 8.7 days and from 1.3 to 4.3 hours, respectively. Based on the results, desmedipham **doesn't degrade rapidly** in soil under aerobic conditions. However, the degradates aniline, EHPC and phenyl urethane showed **rapid dissipation** under the same conditions.

The endpoints are presented in table (Table 40) above and the studies are summarized below. However, since other data (screening and simulation tests) are available and water/sediment or soil fate studies are not among the preferred data to be used for assessing rapid degradability according to the CLP guidance, there is no need for further investigations of the data. These results do not impact the environmental classification.

Aerobic mineralisation in surface water

One biodegradation simulation study (**RAR B.8.2.2.2/01**, **2014** & **B.8.2.2.2/02**, **2014**) was available in the RAR. Aerobic mineralisation of [aniline-UL-^{14}C]-desmedipham in surface water was investigated under defined laboratory conditions in the dark. The study followed the OECD test guideline 309 (2004). The radiolabelled test item was applied water at concentrations of 0.1 and 0.01 mg/L. Additionally, the high concentration experiment was performed under sterile conditions in order to gain information about abiotic degradability of the test item.

Desmedipham dissipated fast in surface water with a half-life of < 1 day, regardless of its concentration. The main degradation product, in both the high and low dose system, was aniline. Subsequently, in the high dose

system diphenyl urea was detected and accounted for 18.0 , 3.2 and 4.0 % of AR (mean values) after 3, 7 and 14 days of incubation, respectively. In addition, the formation of diphenyl urea was observed in the high dose sterile system from day 3 to day 62, with mean values between 3.4 and 17.2 % of AR on day 62. The formation of CO_2 was slightly lower in the sterile condition suggesting the need of microbial degradation for mineralisation.

A kinetic analysis of the residue data of desmedipham and its degradates in surface water was performed following the guidance of the FOCUS Kinetics Work Group, in order to derive half-lives and formation fractions suitable for use in exposure assessment. The model fit as well as the statistical evaluation of the results was carried out with the software KinGUI, version 2.1 with four different kinetic models: SFO (Single First-Order), FOMC (First Order Multi-Compartment), HS (Hockey Stick) and DFOP (Double First-Order in Parallel). The most appropriate kinetic model was selected on the basis of a detailed statistical analysis including visual assessment of the goodness of the fits, chi^2 scaled-error criterion and t-test significance. If it turned out that the degradation of the parent compound desmedipham could not acceptably be fitted with SFO kinetics, the best fitting kinetic model was determined by fitting only the parent data separately to alternative models. Then this model was implemented in the model for the whole degradation scheme which was then fitted to the full data set.

For this study, single first order (SFO) was the most appropriate kinetic model for the aerobic mineralisation of desmedipham. The calculated half-lives for the dissipation of desmedipham in surface water under aerobic conditions in the dark in the laboratory was 0.004 days in the high concentration test systems and 0.12 days in low concentration test systems. The half-lives of aniline were 75.9 days and 34.9 days for high and low concentrations, respectively. The half-life of diphenyl urea could be estimated for high concentration test systems only with a value of 2.7 days.

Water-sediment data

Study 1 – desmedipham

The degradation behaviour of desmedipham was investigated in an aerobic laboratory water-sediment study (**RAR B.8.2.2.3/05**, **1994** & **B.8.2.2.3/06**, **1994)** with [^{14}C-aniline]-desmedipham in two different test systems: a loamy sand (Rhine river) and a clay-loam (Anwil pond). The test was conducted in line with the OECD test guideline 308 (2002). Desmedipham (dissolved in acetone) was applied at a concentration of 160-161 µg/L water. The level of radioactivity in the water phase decreased from 98.4 % of the applied radioactivity on day 0 to 1.2 % on day 105 for Rhine River and from 96.4 to 0.7 % for pond during the same interval. In the sediment, the radioactivity increased from 1.2 % on day 0 to 38.3 % on day 1 and decreased again to 20.5 % on day 105 for Rhine River. For pond, the respective values were 1.9 % (day 0), 36.9 % (day 14) and 32.2 % (day 105). According to the RAR, the fast increase and the subsequent decrease of bound radioactivity probably reflected the fast binding of aniline which was the main metabolite found during the study, followed by its subsequent mineralization. The non-extractable residues, i.e. 11.2 % for the river samples and 25.1 % for the pond samples, were primarily found in the humin fraction.

The recoveries obtained for Rhine River were 99.5 % of applied radioactivity on day 0 and 91.4 % on day 105, whereas the lowest recovery was obtained on day 3 with 74.6 %. The respective values obtained for pond were 98.3 % on day 0 and 90.9 % on day 105 with 74.7% on day 3 representing the lowest recovery. According to the OECD test guideline 308 the recovery should be 90-110 % for the labelled substances. Low recoveries during days 1 to 14 was explained in the RAR to result from high losses of volatiles (mainly $^{14}CO_2$) during processing of the samples. According to RAR, this assumption can be accepted since the later sampling points showed no loss of radioactivity.

The dissipation of desmedipham was extremely fast in both, the river and the pond system since the pH of the water in both test systems were around 8.2. The DT_{50} and DT_{90} values were below one day for both systems. Aniline was formed with mean maximum occurrences in water phase of 55.4 % and 55.1 % and of AR in system Rhine River and Anwil Pond, respectively. Aniline was not found in sediment. Diphenyl urea was formed with mean maximum occurrences in water phase of 4.7 % and 4.4 % and of AR in system Rhine River and Anwil Pond, respectively. The results also show high level of mineralisation, 66.4 and 56.0 % of

AR. The kinetic evaluation followed the FOCUS DegKinetics Report (2006). Not enough data were available for a reliable determination of degradation kinetics at level II.

Study 2 – desmedipham, EHPC and aniline

In the second study (**RAR B.8.2.2.3/07**, **2003** & **B.8.2.2.3/08**, **2003**), the degradation behaviour of desmedipham was investigated in an aerobic laboratory water-sediment test with [aniline-UL-^{14}C]- and [phenoxy-ring-UL-^{14}C]-labelled desmedipham in two different test systems: a sandy-loam (OCR pond) and a clay-loam (CMS pond) from Pennsylvania, USA. The test was performed according to the OECD test guideline 308 (2002). The overall mean recovery of [^{14}C-phenoxy]-desmedipham for both water/sediment systems was good (96.2 % for the OCR Pond and 93.4 % for the CMS Pond). However, the overall mean recovery of [^{14}C-aniline]-desmedipham was less than 90 % at some time points, the recovery tending to decrease over time. The low recoveries were due to the systematic loss of volatile carbon dioxide during processing. This was tested with additional water/sediment samples incubated for 121 days and either NaOH was used to stabilize the $^{14}CO_2$ as $CaCO_3$ or HCl to completely purge the $^{14}CO_2$ from the water.

Desmedipham was rapidly hydrolysed to its main degradation products EHPC and aniline in the water phase. The degradation of desmedipham was faster in OCR pond (DT_{50} 0.13 days; geomean of the two labels) which had higher water pH than in CMS pond (DT_{50} 3.1 days; geomean of the two labels). The respective DT_{90} values were 0.43 and 12.6 days. EHPC was further dissipated with DT_{50} of 50.3 and 64.2 days in total systems of OCR and CMS ponds, respectively. The max amount of EHPC was 95.7 % in water phase and 10.1 % in sediment. Also, aniline was further dissipated with DT_{50} of 5.3 and 42.0 days in total systems of OCR and CMS ponds, respectively. The max amount of aniline was 71.9 % in water phase and 0.6 % in sediment. The CO_2 production at the end of the study ranged from 14.6 % to 43.7 % for CMS and OCR ponds, respectively. The kinetic evaluation followed the FOCUS DegKinetics Report (2006). Not enough data were available for a reliable determination of degradation kinetics at level II for both systems.

Study 3 – desmedipham, aniline and phenol

The third study (**RAR B.8.2.2.3/09**, **2003**) was based on leftover sample material from a previous water/sediment study (B.8.2.2.3/07, 2003) as further attempts were conducted for identification of the 5-minute degradation product found in some aqueous phase samples generated in the CMS system treated with [aniline-UL-^{14}C]-desmedipham. The aqueous phase from sample 135, collected at day 3, was selected for the isolation and identification of a degradate with a 5-minute chromatographic retention time using an ion-pairing HPLC mobile phase as the unknown degradation product was previously-reported to represent approximately 5 % of AR in this sample.

A large chromatographic peak eluting at approximately 4.9 minutes was observed in the total ion chromatograms of the 5-minute degradate and phenol. The mass spectra of the 4.9-minute peak of the degradate and phenol both exhibited major ions at m/z 94 and m/z 66. Based on these results, it the radioactive degradate eluting at 5 minutes was phenol. The kinetic evaluation of the degradation of desmedipham to phenol was not performed according to the FOCUS DegKinetic Report (2006) but the DT_{50} value of 4.3 days (based on the visual fit) is considered acceptable in the RAR.

Study 4 – desmedipham and aniline

In the fourth study (**RAR B.8.2.2.3/10**, **2003**), the aerobic aquatic metabolism of desmedipham was investigated in line with the OECD test guideline 308 (2002) in two fresh water aquatic-sediment systems (Turkey Creek and Choptank River from the USA) at 20 °C in the dark for up to 101 days. The mean material balances for all four test systems were > 90 % of AR. The amount of desmedipham decreased in the water of Turkey Creek test systems from 92.2 to 4.2 % of AR. In Choptank River test systems the amount of desmedipham decreased from 71.5 % to 1.5 % of AR. Desmedipham was rapidly hydrolysed to its main degradate aniline with mean maximum amounts of 8.7 and 11.5 % of AR, decreasing to 0.9 % of AR (day 14) and 0.2 % of AR (day 30) in Turkey Creek and Choptank River test systems, respectively. Aniline was further degraded, but no DT_{50} values could be obtained due to inconsistent residue decline.

The dissipation of desmedipham in total system was fast in both river systems resulting to best fit DT_{50} values of 1.3 and 0.37 days in Turkey Creek and Choptank River, respectively. The maximum amount of desmedipham found in sediment of Turkey Creek desmedipham was detected with amounts ≤ 0.2 % of AR until day 7 in the sediment of Choptank River test systems, desmedipham was detected only in one replicate at day 0 with 11.4 % of AR. The amount of CO_2 increased to 32.5 and 31.8 % of AR at the end of the test showing quite high rate of mineralisation in both systems. The amout of CO_2 in abiotic systems was 1 % of AR showing the importance of microbial degradation for mineralization. The evaluation followed the FOCUS DegKinetics Report (2006). However, it was not possible to calculate level II degradation values for water and sediment phases for aniline labelled desmedipham in either system due to too little data available for sediment.

Study 5 – desmedipham and EHPC

In the fifth study (**RAR B.8.2.2.3/11**, **2003**), desmedipham was incubated aerobically in the laboratory in two non-contaminated water/sediment systems from Oostvaardersplassen (OVP) and Schoonrewoerdsewiel (SW) at 20 ± 2 °C in the dark for 99 days. The study was performed in line with the OECD test guideline 308 (2002). The test substance concentration in the water layer was approximately 63 µg/L per vessel. The overall mean recovery of [^{14}C-phenoxy]-desmedipham for SW was good (92.8 – 105.4 % of AR) but below the range for OVP once on day 20 (OVP; 85.9-113.3 % of AR). Since the mean recovery returned to > 90 % of AR for the rest of the sampling days the mean recovery can be considered acceptable.

Desmedipham hydrolysed from the water layer in both water/sediment systems in less than 0.06 days. Apart from EHPC, no significant metabolites were observed. Hydrolysis of desmedipham from the water layer resulted to the formation of EHPC, followed by transfer of EHPC to the sediment and mineralisation. No desmedipham was found in the sediment extracts. EHPC represented up to 88 % of AR (OVP) and 90 % of AR (SW) in the water/sediment systems. The sum of unknowns in the water layers never exceeded 12 % of AR. Mineralisation was an important degradation process in the both systems resulting the amount of CO_2 increasing to 36 % of AR and 29 % of AR after 99 days in OVP and SW test systems, respectively. Dissipation of EHPC from the sediment was the result of mineralisation and formation of bound residues. The amount of unextracted residues increased gradually to 71 % of AR (OVP) and 69 % of AR (SW) after 99 days of incubation. The kinetic evaluation followed in other aspects the FOCUS DegKinetics Report (2006) but only single samples were analysed in each sampling date. Although there was no replication, the study is still considered acceptable.

Study 6 – desmedipham and EHPC

The objective of sixth study (**RAR B.8.2.2.3/12**, **2003**) was to determine the degradation of [phenoxy-ring-UL-^{14}C]-desmedipham in two water/sediment systems under aerobic conditions in the dark for 121 days at 20 °C (± 2 °C). The study was performed in line with the OECD test guideline 308 (2002). The mean total recovery was 98.4 % of AR and ranged from 82.5 to 108.3 % of AR and from 88.3 to 106.9 % of AR in Illingen pond and Dentelbach creek systems, respectively. In both systems the radioactivity was once below 90 % of AR. The non-volatile radioactivity in water decreased with time in the water phase to 2.8 and 5.0 % of AR for pond and creek test systems, respectively. $^{14}CO_2$ accounted for around 20.4 and 28.8 % of AR at day 121 in pond and creek test systems, respectively. No organic volatiles were trapped. Radioactivity in sediment of pond test system increased to 68.1 % of AR (62.8 % of AR being non- extractable bound residues) and to 58.1 % of AR (55.1 % of AR being non-extractable bound residues) in pond and creek test systems, respectively.

The water phases of both systems were alkaline and desmedipham hydrolysed rapidly in both systems and hence no desmedipham was found in sediment. Dissipation of desmedipham from the water layer resulted to EHPC, followed by transfer of EHPC to the sediment and mineralisation. 91.3 and 96.3 % of AR was found as EHPC at day 1, degrading further to 7.6 and 7.7 % of AR at day 121 in the total system of pond and creek test systems, respectively. The major compounds observed in the sediment were EHPC (14.9 and 9.2 % of AR at day 30 for pond and creek test systems, respectively) and the bound residues (63.4 and 55.1 % of AR at day 62 for pond and creek test systems, respectively). The obtained SFO DT_{50} values for desmedipham for

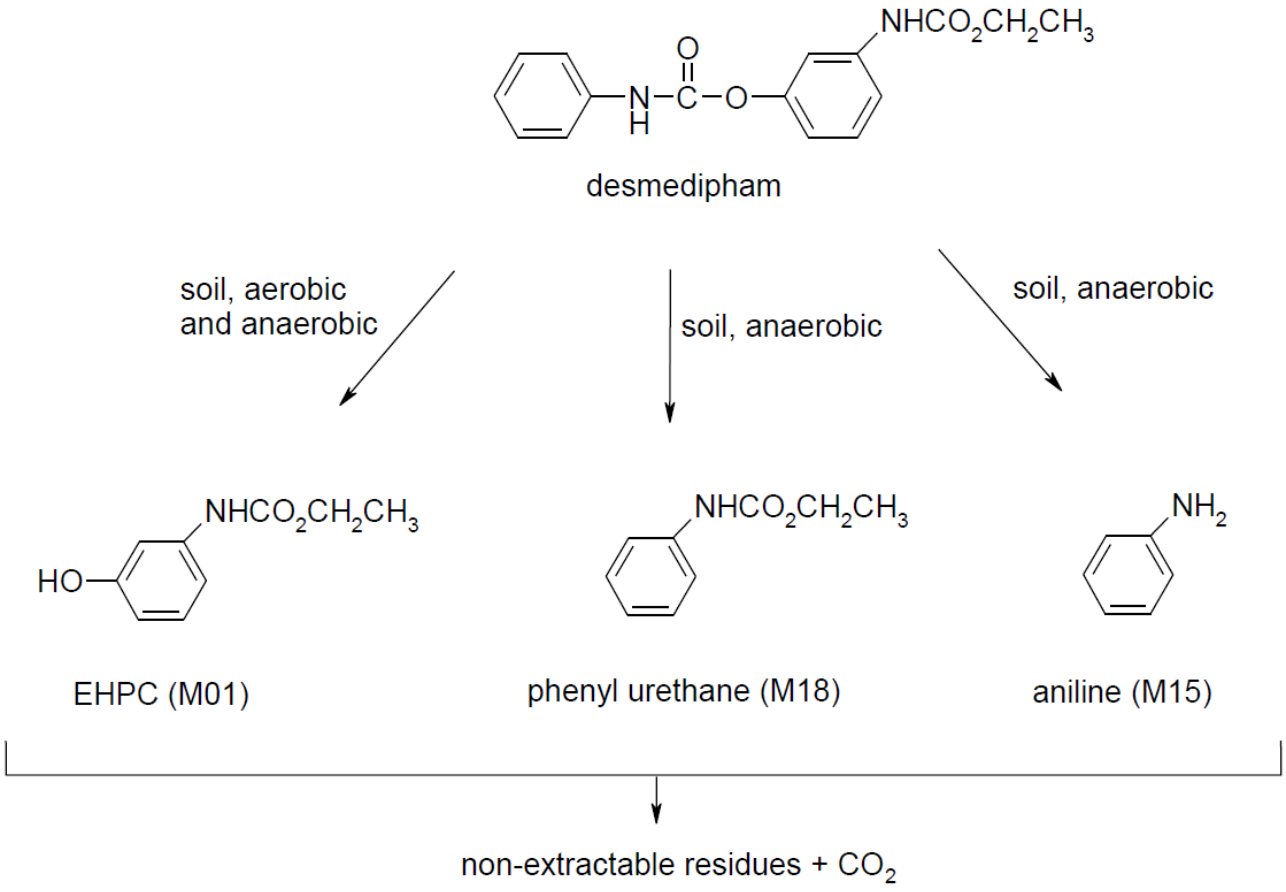
trigger evaluation were 0.052 and 0.050 days for Illingen pond and Dentelbach Creek, respectively. For EHPC, a SFO DT_{50} values of 10.1 and 23.4 days was obtained for trigger evaluation in Illengen pond and Dentelbach Creek, respectively. The kinetic evaluation followed the FOCUS DegKinetics Report (2006).

Aerobic degradation in soil

Study 1 – desmedipham

Soil degradation studies were assessed to address the degradation scheme for desmedipham in soil. In this comparative study (**RAR B.8.1.1.1.1/01**, **2014**), fourteen laboratory studies were assessed and of these studies, nine were deemed suitable for analysis with 15 soils. The proposed pathway scheme (**Figure 2**) is presented below.

Figure 2. Proposed pathway scheme for the degradation of desmedipham in soil.



Study 2 – desmedipham and EHPC

The degradation of [phenoxy-ring-U-^{14}C]-desmedipham in was studied (**RAR B.8.1.1.1.1/06**, **1991**) in the dark in German Standard Soil 2.3 for up to 100 days at 21 °C. The study was basically performed in line with the OECD test guideline 307 (2002) except no replicates were included in the study. The application rate was 2.9 mg a.s./kg and a separate study was performed in sterile soil. Desmedipham was rapidly degraded into EHPC and other lesser unidentified breakdown products which were further degraded and mineralised to CO_2. The degradation of desmedipham in sterile soil was much slower. The total radioactive recovery was above or close to 90 % which fulfils the quality criteria in OECD TG. The kinetic evaluation followed the FOCUS DegKinetics Report (2006). The non-normalised SFO DT_{50} value of 8.8 days was obtained. For EHPC, no acceptable fit could be obtained.

Study 3 – desmedipham

The degradation rate of [aniline-UL-^{14}C]- and [phenoxy-ring-UL-^{14}C]-labelled desmedipham was studied (**RAR B.8.1.1.1.1/07**, **1991**) in the dark in 4 soils (Speyer 2.2, a sandy loam, acidic clay loam and alkaline clay loam) for up to 100 days at 21 °C, at 40 % of maximum water holding capacity (MWHC). The application rate was 918.4 µg a.s./kg. The study was partly performed in line with the OECD test guideline 307 (2002): no LOD and LOQ of the analytical method was given, soil sampling and storage of the soil was not described, no use history of desmedipham at the field site was given and microbial activity was not measured.

The total radioactive recovery was close to 100 % which fulfils the quality criteria in OECD TG. Degradates diphenyl urea and EHPC were analysed. Less than 2 % of AR was found as EHPC. An unidentified metabolite was found max 2.6 % of AR. The kinetic evaluation followed the FOCUS DegKinetics Report (2006). The non-normalised best fit DT_{50} values of 28.2, 25.1, 33.1 and 8.0 days were obtained in Speyer 2.2, a sandy loam, acidic clay loam and alkaline clay loam, respectively.

Study 4 – desmedipham and EHPC

The degradation of [phenoxy-ring-UL-^{14}C]-desmedipham was studied (**RAR B.8.1.1.1.1/08**, **1993**) in the dark in German Standard Soil 2.1 for 28 days at 20 °C, at 40 % of MWHC. The application rate was 0.54 mg a.s./kg. The study was basically performed in line with the OECD test guideline 307 (2002) but following deviations were noted: only one sample was taken at each sampling time and use history of desmedipham at the field site was not described.

The total mean radioactive recovery was 96.1 % (± 2.1 % standard deviation) which fulfils the quality criteria of 90-110 % of AR in OECD TG. The kinetic evaluation followed the FOCUS DegKinetics Report (2006). The non-normalised best fit DFOP DT_{50} value of 3.7 days and for modelling purposes a pseudo SFO DT_{50} value of 28.2 days were obtained for desmedipham in German Standard Soil 2.1. For trigger and modelling evaluation, a non-normalised SFO DT_{50} value of 1.4 days was obtained for EHPC.

Study 5 – EHPC

The rate of degradation of [^{14}C-phenyl]-EHPC was studied (**RAR B.8.1.1.1.2/02**, **2003**) in the dark in three soils for 61 days at 20 °C, at 50 % of MWHC. The application rate was 0.048 mg a.s./kg. The study was performed in line with the OECD test guideline 307 (2002), but the following deviations were noted: the biomass was below 1 % for soil #3 of OC at both the initial and final timepoint and it was below 1 % for soil #1 at the initial time point, use history of desmedipham at the field sites was not described, only a single sample was taken at each sampling time and LOD and LOQ of the analytical method were not included.

The total mean radioactive recovery was 97.9 ± 4.0 %, 96.7 ± 2.9 % and 93.4 ± 3.4 % in sandy loam 1, sandy loam 2 and clay loam, respectively, which fulfils the quality criteria of 90-110 % of AR in OECD TG. Also, the pH and orgC were within the recommended limits of the OECD TG, except for clay loam which has higher orgC content (2.9 %). However, since the range is only a recommendation the study is considered valid. The kinetic evaluation followed the FOCUS DegKinetics Report (2006). The best fit DT_{50} value of 0.07 (FOMC), 0.26 (SFO) and 0.39 days (SFO) were obtained for EHPC in sandy loam 1, sandy loam 2 and in clay loam, respectively.

Study 6 – EHPC

The rate of degradation of phenyl labelled EHPC was studied (**RAR B.8.1.1.1.2/03**, **1997**) in three soils under aerobic conditions in the dark in the laboratory for up to 61 days at 20 °C and at field capacity. An amount of 0.0248 mg EHPC/100 g soil (dry weight) was used. The study was performed in line with the OECD test guideline 307 (2002) with two deviations noted: only one sample was taken at each sampling

time and the use history of desmedipham at the field sites was not described. The study is still considered valid.

The mean radioactive recovery was 93.0, 93.0 and 91.0 % in Speyer 2.1, Speyer 2.2 and Speyer 2.3, respectively, which fulfils the quality criteria of 90-110 % of AR in OECD TG. Also, the microbial biomass was above 1 % of the total organic carbon and the pH and orgC were within the recommended limits of the OECD TG. The amount of EHPC decreased from 67.8, 63.7 and 48.6 % of AR at day 0 to 4.3, 1.2 and 3.4 % of AR at day 14 for soils Speyer 2.1, Speyer 2.2 and soil Speyer 2.3, respectively. From the radio-TLC analyses, it can be concluded that EHPC was the only radioactive component that is present in the soil extracts. The kinetic evaluation followed the FOCUS DegKinetics Report (2006). The best fit SFO DT_{50} value of 3.5 and 1.0 days were obtained for EHPC in Speyer 2.2 and Speyer 2.3, respectively. Due to originally incorrect value, DT_{50} value of 8.7 days was obtained after re-performing the kinetic evaluation of dissipation of EHPC for soil Speyer 2.1.

Study 7 – aniline

The effects of low temperature and accelerated soil-solution contact on soil adsorption of labile organic chemicals using aniline were studied (**RAR B.8.1.1.1.2/05**, **1987**). The tests were conducted using silt loam (West Virginia, USA). The study does not follow any guidelines for aerobic degradation in soil. The kinetics of adsorption and degradation of aniline (^{14}C-labelled) in soil solution phase at 3 and 22 °C was studied. Two mathematical models were evaluated for describing the radioactivity disappearance data.

In this summary only, the degradation of aniline in soil suspension at 22 °C is reported. An overall rate coefficient for the disappearance pattern of aniline at 22 °C was calculated (model 1). This value of 3.577 x 10^{-4} has been transformed to the DT_{50} value of 1.35 days. In another model (model 2) a larger degradation rate coefficient was predicted. This value of 2.69 x 10^{-2} has been transformed to a DT_{50} value of 0.018 days. Aniline was not found in any of the soil aerobic studies performed with [aniline-UL-^{14}C]-labelled desmedipham. Aniline is formed due to the hydrolysis of desmedipham, but the results from the aerobic degradation studies suggest that aniline is very quickly degraded further. According to the RAR, the results from this study support this conclusion.

Study 8 – phenyl urethane

The rate of degradation of unlabelled phenyl urethane was studied (**RAR B.8.1.1.1.2/06**, **2014** & **B.8.1.1.1.2/07**, **2014**) in four soils under aerobic conditions in the dark in the laboratory for up to 48 hours at 20 °C. An amount of 13 µg phenyl urethane/kg soil (dry weight) was used. The study was performed in line with the OECD test guideline 307 (2002), except that open systems were used and therefore volatiles could not be collected. The mean recovery was 110 ± 2 , 104 ± 1 and 105 ± 1 % in Laacher Hof AXXa, Hoefchen am Hohenseh and Dollendorf, respectively, which fulfils the quality criteria of 90-110 % in OECD TG. For soil Laacher Hof Wurmwiese the mean recovery was 121 ± 9 % at time zero. In all four soils the test item decreases from 105-121 % at 0 hours to 49-65 % after 4 hours. In case of soil Dollendorf (clay loam) the initial concentration of 105 % at 0 hours decreased to 13 % after 4 hours. A constant decline to rather constant value of 3 to 6 % from 4 hours onwards until the end of the incubation period at 48 hours could be observed. In case of soil Dollendorf the concentration from 4 hours onwards until 48 hours declined down to 0.4 %. The fast decline of the test item in soil Dollendorf (DT_{50} value of 1.3 hours and a DT_{90} of 4.4 hours) could be attributed to an organic carbon content of 4.7 % being more than twice as high as in the other three soils. A mass balance could not be calculated because an open system was used.

The kinetic evaluation followed the FOCUS DegKinetics Report (2006). Measured and reported true replicates were taken into account singularly. The half-lives of phenyl urethane under aerobic conditions were 4.5, 3.8, 1.3 and 4.3 hours in soils Laacher Hof AXXa, Hoefchen am Hohenseh, Dollendorf and Laacher Hof Wurmwiese, respectively. The DT_{90} values in these soils are 15.0, 12.6, 4.4 and 22.5 hours for soils Laacher Hof AXXa, Hoefchen am Hohenseh, Dollendorf and Laacher Hof Wurmwiese, respectively. Primary degradation of phenyl urethane was relatively fast under aerobic conditions in the environment. According to the RAR, results obtained confirmed that phenyl urethane was mainly subjected to a biological

degradation under aerobic conditions at ambient temperature as sterile controls confirmed absence of abiotic decline of concentration.

11.1.4.3 Photochemical degradation

Six studies on photochemical degradation in water for desmedipham and one for metabolite EHPC conducted generally according to the OECD test guideline 318 "Photo transformation of Chemicals in Water – Direct Photolysis" (or older test version) were considered valid in the RAR. The direct photolysis of desmedipham was shown to be insignificant **as no photodegradation occurred** after several days of continuous exposure at pH 4-5. Metabolite EHPC, the primary hydrolysis degradate known to be stable for hydrolysis, was **degraded** due to photolysis and a DT_{50} value of 9.2 days was determined.

Also soil and air photolysis studies are available in the RAR. The studies following the OECD test guideline draft "Photo transformation of Chemicals on Soil Surfaces" show the soil photolysis being relatively slow reaction with DT_{50} values from 286.4 to 566.1 days. Desmedipham entering the air is subject to **rapid** indirect photochemical **degradation** (DT_{50} value of 10.8 hours for hydroxyl radical reaction).

The endpoints are presented in table (Table 40) above and the studies are summarized below. However, since other data (screening and simulation tests) is preceding over photolysis data for classification purposes, there is no need to investigate the data further. Therefore, detailed description of these field studies is excluded from this CLH report.

Photodegradation in water

Study 1 – desmedipham

The photolysis of desmedipham in water was studied (**RAR B.8.2.1.2/02**, **1992**) according to the US EPA guideline: Pesticide Assessment Guidelines: Subdivision N: Photodegradation studies in water (1982). The study showed with a 0.01 g/L solution of desmedipham that desmedipham was not degraded after exposure to simulated daylight for 15 days at 25.0 ± 1 °C at buffer solution of pH 4.

Study 2 – desmedipham

The second study (**RAR B.8.2.1.2/03**, **1992**) was performed in line with OECD test guideline 316 (2008) in compliance with GLP. The amount desmedipham in dark controls decreased from 100 to 98.5 % of AR after 18 days. According to the RAR, this can be explained by the slow hydrolysis rate of desmedipham in pH 4. No degradation of desmedipham occurred in irradiated samples, since the amount of desmedipham after 18 days was still 103 %. The results are in line with the previous study.

Study 3 – desmedipham

The third study (**RAR B.8.2.1.2/04**, **1992**) was performed according to EPA Provisional Chemical Fate Guidelines 795.70 (1988) and OECD Draft TG: Photo transformation of chemicals in water, part A (1990) and in compliance with GLP. Photodegradation was only observed in the photolysis solutions in synthetic natural water, pH 4. No degradation occurred in the photolysis solutions in distilled water, pH 4 and in the dark control solutions which is in line with the previous study. Therefore, the decay of desmedipham observed in the synthetic natural water solutions is caused by indirect (sensitized) photodegradation only i.e. the substance could undergo slow photodegradation in natural waters. Based on first order kinetics, the half-life was calculated to be 106 hours in synthetic natural water with 95 % confidence.

Study 4 – desmedipham

The direct photolysis of desmedipham in water was studied (**RAR B.8.2.1.2/06**, **2004**) according to the OECD test guideline 316 (2008). For [phenoxy-ring-UL-^{14}C]-desmedipham significant first order degradation curves could be fitted through the data at pH 5 and at pH 7 in exposed solutions and dark

controls. Evaluation of the kinetic data revealed no indication of photolysis at pH 5 and at pH 7. For [aniline-UL-^{14}C]-desmedipham no photolysis was observed in buffer of pH 5. For pH 7, significant first order degradation curves could be fitted through the data in exposed solutions and dark controls but evaluation of the kinetic data revealed no indication of photolysis at pH 7.

Determination of photolysis at pH 7 and 9 was severely hampered by hydrolysis. The results with buffer solutions with pH 7 and pH 9 showed high rate of hydrolysis which was at equal level in irradiated and dark samples both with [aniline-UL-^{14}C]- and [phenoxy-ring-UL-^{14}C]-labelled desmedipham. Therefore, the study suggests desmedipham being photolytically stable.

Study 5 – desmedipham & EHPC

The photolysis of [phenoxy-ring-UL-^{14}C]-desmedipham in natural water was investigated (**RAR B.8.2.1.3/01**, **2004**). The study followed the OECD test guideline 316 (2008). Desmedipham was rapidly degraded in natural water at 25 °C and there was no significant difference between the rate of degradation in the light and in the dark. The recovered radioactivity in all cases was above 90 % (range 90.3 to 103.4 %). DT_{50} and DT_{90} values were calculated by applying a first order kinetic model. The DT_{50} values for desmedipham in the irradiated experiments and irradiated experiments were 0.17 and 0.57 hours (corresponding to 1.26 and 4.22 hours under solar conditions in Tokyo, Japan), respectively. The corresponding DT_{90} values in the irradiated and non-irradiated experiments were 0.27 and 0.89 hours (corresponding to 2.00 and 6.59 hours under solar conditions), respectively.

The hydrolysis product of desmedipham was EHPC in both the irradiated and non-irradiated experiments. EHPC is known to be stable for hydrolysis and hence the study can be used to assess the indirect photolysis of EHPC. EHPC was stable under dark conditions but was further degraded in the light giving rise to numerous photolysis products. The major photolysis product of EHPC was carbon dioxide (up to 38 % of AR). In addition, one component, characterised as having molecular weight 123 but not identified despite various efforts made, was formed at a concentration > 10 %. The DT_{50} value of EHPC was 9.2 days and the DT_{90} value was 30.6 days (both Tokyo spring daylight equivalents).

Photodegradation in water and quantum yield

The degree of photolytic degradation and the quantum yield of desmedipham were determined (**RAR B.8.2.1.2/07**, **2002**). Light absorption of desmedipham was determined by recording spectra in buffer solutions of different pH (5, 7 and 9) and different acetonitrile concentrations (10, 50 and 100 %). The study followed the OECD test guideline 316 (2008). There was no dependency of the UV/VIS-spectra of desmedipham on pH or co-solvent concentration observed in the relevant wavelength range.

For the irradiations two different kinds of ^{14}C-labelled desmedipham were used in order to detect all known degradation products, if they appeared. A mean rate constant kD of $1.27*10^{-6}$ s^{-1} was determined assuming a pseudo-first order kinetics for the photodecomposition process. This means a calculated half-life of about 6.4 days on experimental conditions. As a major product of photolysis EHPC was identified, amounting up to 19 % of AR. Further transformation products, which amounted to more than 10 % of AR, were observed by ^{14}C-detection but could not be identified.

Based on the degradation rate constants and the molar absorption coefficients the quantum yield for direct photolysis was calculated according to the OECD draft test guideline. A low mean quantum yield of Φ = 0.00146 was calculated on the basis of UV adsorption data and the degradation kinetics determined from the photodegradation study. The quantum yield is characteristic for a molecule and independent of the type of the light source and of the concentration of the target compound. Therefore, extrapolations to environmental scenarios and different concentrations are possible.

Environmental half-lives were predicted using a computer program based on a model developed by Frank and Klöpffer. The molar absorption coefficients determined and the calculated quantum yield were used as input and no dissipation processes other than photolysis were considered. The calculation was performed for pure water. The calculated environmental half-lives of sunlight exposed top surface water layers (0-10 cm) at 52° North were between 10 days and 2.8 years, depending on the solar irradiance intensity at the respective

months. The estimated half-life varies between 15.6 to 22.0 days for a direct photodegradation during periods of main use in spring and summer. The shortest half-life was calculated for June with 10.7 days (medium values, assuming normal climate conditions).

Photodegradation in soil

Study 1 – desmedipham

The photolytic route and rate of degradation of [^{14}C]-desmedipham (separately labelled in the aniline and phenoxy ring) were studied (**RAR B.8.1.1.3.1/04**, **2003**). The study was performed according to the OECD draft test guideline "Photo transformation of chemicals on soil surfaces" (January 2002) but with a differing temperature range (20 ± 5 °C instead of 20 ± 2 °C). Test concentrations of 47.2 µg per test system were applied. The mean recovery of applied radioactivity for both labels was acceptable (^{14}C-aniline: 90.2 ± 2.4 % and ^{14}C-phenoxy: 92.9 ± 3.3 %). The degradate EHPC was found once; 4.5 % of AR. Aniline was not detected in this study. Due to limited number of sampling points (n=3) for aniline labelled desmedipham no kinetic evaluation was performed for the dark control and hence the effect of photolysis could not be evaluated for aniline labelled desmedipham.

The kinetic evaluation was performed according to the FOCUS DegKinetics (2006). SFO DT_{50} values of 37.9 and 78.3 days were obtained in irradiated and dark controls, respectively, for phenoxy labelled desmedipham in the test system used and hence the DT_{50} values indicate faster degradation in irradiated samples compared to dark controls. A DT_{50} value of 286.4 days for soil photolysis (dark minus irradiated) was obtained at summer sunlight at 30° to 50° North

Study 2 – Desmedipham

The photolytic route and rate of degradation of [^{14}C]-desmedipham (separately labelled in the aniline and phenoxy ring) were studied (**RAR B.8.1.1.3.1/05**, **2004**) on one soil under irradiation. The study was performed according to the OECD draft test guideline "Photodegradation of chemicals on soil surfaces" (January 2002), except that the temperature was higher (24 ± 2 °C) for the irradiated samples than recommended (20 ± 2 °C) in OECD TG. The application rate of the phenoxy labelled desmedipham was 0.221 mg/kg soil and for the aniline label 0.185 mg/soil (dry weight), respectively.

The mean recovery of applied radioactivity for both labels were also acceptable ([phenoxy-ring-UL-^{14}C]-desmedipham: 93.3 ± 5.0 % and [aniline-UL-^{14}C]-desmedipham: 98.0 ± 4.1 %). The evaluation of the degradation was performed according to the FOCUS DegKinetics Report (2006). DT_{50} values of 439.7 and 566.1 days for phenoxy and aniline labelled desmedipham for soil photolysis was obtained at summer sunlight at 30° to 50° North, respectively.

Photodegradation in air

The photochemical degradation of desmedipham in the atmosphere was estimated (**RAR B.8.3.1/01**, **1992**) with the Atkinson method. The estimation of its atmosphere half-life was based on the estimation of the bimolecular OH radical rate constant. According the incremental method of Atkinson it was estimated to be > 3.58 x 10^{-11} cm^3 $molecule^{-1}s^{-1}$. Assuming the global 24-hour average for the concentration of OH radicals to be 5 x 10^5 molecules/cm^3, this value corresponds to a maximum half-life of 10.8 h for the photochemical-oxidative degradation of desmedipham in air.

11.1.5 Conclusion on rapid degradability

A **ready biodegradability test** (OECD test guideline 301D "Closed-Bottle-Test") showed 21 % of desmedipham degrading after 28 days. This indicates desmedipham being not readily biodegradable as the pass level criteria of ready biogradation test (70 % of DOC removal or 60 % of theoretical oxygen demand) within 28 days was not reached.

Based on the **simulation test in surface water** (OECD test guideline 309 "Simulation biodegradation test"), the calculated half-lives for desmedipham ranged from 6 minutes to 3 hours in natural surface water systems. For degradate diphenyl urea, DT_{50} value of 2.7 days was obtained. However, DT_{50} values for degradate aniline were from 34.9 to 75.9 days.

According to **hydrolysis tests** (OECD test guideline 111 "Hydrolysis as a function of pH"), desmedipham is hydrolytically stable under acidic conditions (DT_{50} from 4884 to 351 days at pH 4) but the hydrolytic degradation will be rapid (DT_{50} from 14 minutes to 26 hours at 20 °C) in neutral and alkaline (pH 6-9) environments such as many natural waters. However, the test results also suggest that the desmedipham degradation products, aniline and EHPC, do not hydrolyse as their concentration increased towards the end of the studies.

The studies on degradation of desmedipham in **water/sediment systems** support the beforementioned observations for desmedipham and its degradation products. Based on the degradation results of **soil degradation** studies, desmedipham DT_{50} ranged from 3.7 to 127.2 days in soil under aerobic conditions. In the **inherent biodegradation test (**OECD test guideline 302C "Modified Miti-test (II)"), 45 % degradation of desmedipham was achieved after 28 days. Furthermore, **photodegradation** of desmedipham was measured being insignificant in water and soil. For the degradate EHPC, a photodegradation DT_{50} value of 9.2 days was determined in water.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for the classification proposal.

11.3 Environmental fate and other relevant information

A brief summary of relevant studies on environmental fate, listed in the Renewal Assessment Report (RAR), is reported below. Only the information considered adequate, reliable and relevant for the classification proposal of desmedipham has been included.

Table 41: Summary of relevant information on environmental fate and other relevant information

Method	Results	Remarks	Reference
Environmental distribution			
Adsorption/desorption – desmedipham			
OECD TG 106: Adsorption – Desorption Using a Batch Equilibrium Method (2000) [aniline-UL-^{14}C]-desmedipham (specific activity: 3.91 MBq/mg and radiochemical purity ≥ 98 %) GLP compliant	**Desmedipham adsorption constants** in **Soil type** **K_f*** (mL/g) **K_{oc}** (mL/g) **1/n** **AX** 99.49 5236 0.824 **HF** 77.39 4300 0.818 **HN** 140.97 5035 0.829 **WW** 86.92 4139 0.849 **DD** 95.44 1909 0.869 *K_f = Freundlich adsorption coefficient **Desmedipham desorption constants** in **Soil type** **K_f** (mL/g) **K_{oc}** (mL/g) **1/n** **AX** 150.30 7910 0.836 **HF** not evaluated* **HN** 187.89 6710 0.841 **WW** 124.09 5909 0.861	The mobility of desmedipham in soil based on **K_F** and/or **K_{OC}** values can be classified as **immobile** for adsorption and desorption.	2012 RAR B.8.1.3.1.1/04 M-443682-01-1

Method	Results	Remarks	Reference
	DD not evaluated* * test item not regarded stable enough in soil		
Adsorption/desorption – degradate EHPC			
OECD TG 106: Adsorption – Desorption Using a Batch Equilibrium Method (2000) * [phenyl-UL-^{14}C]-EHPC (specific activity: 618 MBq/mmol and radiochemical purity > 97.4 %) GLP compliant	**Desmedipham adsorption constants** in **Soil type** **K_f*** (mL/g) **K_{oc}** (mL/g) **1/n** **Soil I** 0.854 81.7 0.749 **Soil II** 1.68 160.5 0.782 **Soil III** 0.617 59.1 0.781 **Soil IV** 0.352 43.3 0.888 *K_f = Freundlich adsorption coefficient **EHPC desorption screening test results in** **Soil I** 25.9 % **Soil II** 50.6 % **Soil III** 56.8 % **Soil IV** 81.2 %	Four test soils of European origin (The Netherlands) were used, considered representative for agricultural soils and differing in their physico-chemical properties.	1997 RAR B.8.1.3.1.2/03 M-493974-01-1
Adsorption/desorption – degradate aniline			
Method not specified Non GLP	**K_{oc} value** of 410 mL/g (Kom = 238 mL/g) was **empirically derived.**	In the European Union Risk Assessment Report of Aniline the adsorption behaviour of aniline was summarised based on several publications.	2004 RAR B.8.1.3.1.2/04 M-492497-01-1
Adsorption/desorption – degradate phenyl urethane			
OECD TG 106: Adsorption – Desorption Using a Batch Equilibrium Method (2000) Unlabelled phenyl urethane (chemical purity 99.9 %) GLP compliant	**Phenyl urethane adsorption constants** in **Soil type** **K_f*** (mL/g) **K_{oc}** (mL/g) **1/n** **AXXA** 1.634 90.8 0.838 **HH** 2.147 102.2 0.802 **DD** 6.217 132.3 0.852 **WW** 1.193 56.8 0.950 *K_f = Freundlich adsorption coefficient	The **adsorption behaviour** of **phenyl urethane** (N-phenyl carbamic acid ethyl ester) was studied in four sterilized (γ-irradiated) soils: **AXXA** = sandy loam (Monheim) **HH** = silt loam (Burscheid) **DD** = clay loam (Blankheim) **WW** = sandy loam (Monheim)	2014 RAR B.8.1.3.1.2/05 M-503279-01-1
Volatilisation			
Laboratory volatilisation studies			
OECD TG 104: Vapour Pressure Curve: Vapour Pressure Balance (1981) Desmedipham technical (chemical purity 99.6 %) GLP compliant	**Vapour pressure** of **desmedipham:** 1 x 10^{-8} Pa at 20 °C 4 x 10^{-8} Pa at 25 °C 1 x 10^{-7} Pa at 30 °C	Based on the low vapour pressure, **no significant volatilisation** is expected.	1990 RAR B.2.2/01 M-146570-01-1
Henry's law constant	5.4 x 10^{-7} Pa m^3 mol^{-1} (in distilled water, final pH =	Based on the low Henry's law constant, **no significant**	2012

Method	Results	Remarks	Reference
Desmedipham technical (chemical purity 99.6 %) Non GLP	6.2) at 20 °C	**volatilisation** is expected.	RAR B.2.2/02 M-443774-01-1
Plant and soil volatilisation studies			
BBA Guideline Part IV. 6-1: Testing the volatility behaviour and the fate of plant protection products in the air (1990) [phenoxy-UL-^{14}C]-desmedipham (specific activity: 50.17 µCi/mg and radiochemical purity > 99 %) GLP compliant	Amount of **test substance recovered** after **24 hours** from: **soil samples:** 102 ± 11 % **plant samples:** 97 ± 1.7 %	Less than 20 % of the test substance evaporated in both experiments within 24 hours under the test conditions.	1994 RAR B.8.3.1/02 M-147033-01-1
BBA Guideline Part IV. 6-1: Testing the volatility behaviour and the fate of plant protection products in the air (1990) [phenoxy-UL-^{14}C]-desmedipham (specific activity: 50.17 µCi/mg and radiochemical purity > 99 %) GLP compliant	Amount of **test substance recovered** after **24 hours** from: **soil samples:** 91.2 ± 9.3 % **plant samples:** 99.2 ± 4.0 %	Compared with the previous study, the higher application amount and lower relative humidity did not increase the volatility of desmedipham.	1995 RAR B.8.3.1/03 M-147029-01-1
BBA Guideline Part IV. 6-1: Testing the volatility behaviour and the fate of plant protection products in the air (1990) [phenoxy-UL-^{14}C]-desmedipham (specific activity: 370 kBq/mg and radiochemical purity > 94.3 %) GLP compliant	**91 %** of test substance was r**ecovered** after **24 hours** from plant leaves which equals **volatilisation rate** of **9 %**.	Compared with the previous studies, the lower application amount did not increase the volatility of desmedipham.	2005 RAR B.8.3.1/04 M-494053-01-1

[*] According to the RAR, the study was conducted generally in line with the test method.

11.3.1 Summary of data/information on environmental fate and other relevant information

In the RAR, four studies on adsorption and desorption in soils were considered valid for desmedipham and its degradation products. Desmedipham is considered being immobile for adsorption and desorption in soil based on K_{OC} (mean 4123.6 mL/g) values. Considering the mean K_{OC} values of 86.2 mL/g and 94.6 mL/g

measured from degradates EHPC and phenyl urethane, respectively, it is assumed that the degradates are mobile to moderately mobile in the tested soils. Also, degradate aniline is assumed being moderately mobile with highest K_{OC} value of 410 mL/g.

Based on the very low vapour pressure (1×10^{-8} Pa, 20 °C) and low Henry´s law constant (5.4×10^{-7} Pa m^3 mol^{-1}), desmedipham is virtually non-volatile and, therefore, significant exposure to air is not to be expected. However, there are also three field volatilization studies on desmedipham were provided in the RAR. Desmedipham was applied at rate of 0.125 – 1.0 kg/ha at relative humidity 24 – 50 %. Less than 20 % of the test substance evaporated within 24 hours under the test conditions.

The endpoints are presented in table (Table 41) above and the studies are summarized below. However, these results do not impact the degradation classification. Therefore, no further investigations of the data is needed.

Environmental distribution

Adsorption/desorption

Study 1 – desmedipham

The adsorption/desorption behaviour of [^{14}C-UL-aniline]-desmedipham was studied (**RAR B.8.1.3.1.1/04**, **2012**) in the five different German soils: Laacher Hof AXXa (AX, sandy loam), Hoefchen am Hohenseh (HF, silt loam), Hanscheiderhof (HN, silt loam), Dollendorf II (DD, loam) and Laacher Hof Wurmwiese (WW, sandy loam) using the batch equilibrium method. The study was performed in line with OECD test guideline 106 (2000) and US EPA: OPPTS 835.1230 (2008). The recovery of the applied radioactivity for all concentrations and soils was in the range of 88.8 to 94.3 % of AR (mean 92.5 % of AR).

The adsorption parameters were calculated using the Freundlich adsorption isotherm. The adsorption constants $K_{F(ads)}$ of desmedipham for the five test soils calculated based on the Freundlich isotherms ranged from 77.393 to 140.965 mL/g (mean 100.040 mL/g). The respective $K_{OC(ads)}$ values were in the range of 1908.7 and 5236.3 mL/g (mean 4123.6 mL/g). The Freundlich exponents 1/n were in the range of 0.8177 to 0.8689 (mean 0.84).

Study 2 – EHPC

The adsorption/desorption behaviour of phenyl labelled EHPC was determined (**RAR B.8.1.3.1.2/03**, **1997**) in four soils of European origin (The Netherlands) in the dark using the batch equilibrium method. The study was performed in line with OECD test guideline 106 (2000). The parental mass balances were within the range of 97 to 102 % of AR in soils.

The adsorption constants $K_{F(ads)}$ of EHPC for the four test soils calculated based on the Freundlich isotherms ranged from 0.352 to 1.68 mL/g with corresponding values related to organic carbon ($K_{oc(ads)}$) to range from 43.3 to 160.5 mL/g (arithmetic mean: 86.2 mL/g). Values for the Freundlich exponent of adsorption 1/n ranged from 0.749 to 0.888. Desorption of EHPC was evaluated during the screening test. The percentage of desorption ranged from 25.9 to 81.2 % and from 18.8 to 74.1 % were not desorbed.

Study 3 – aniline

In the European Union Risk Assessment Report of Aniline (**RAR B.8.1.3.1.2/04**, **2004**), the adsorption behaviour of aniline was summarised based on several publications. In the report, an empirically derived K_{oc} value of 410 mL/g is reported which is used in PEC calculations. This is based on a study (Pillai et al., 1982) described as following: in a distribution experiment with radiolabelled aniline (6 concentrations, 0.0317-10 ppm), the radioactivity was measured in the supernatant water phase and the Freundlich adsorption constants were determined. Equilibrium was reached in nonsterile soils within 60 h, but was not attained in sterile soils by 120 h. With 2 nonsterile soils K_{oc} values of 310 resp. 910 mL/g were calculated, while the values decreased to 130 resp. 410 mL/g when the same soils were autoclaved before the experiment. Aniline is degraded partially before adsorption, and the distribution constants for the degradation products

(azobenzene, azoxybenzene and phenazine) are much higher; therefore, the constants determined in nonsterile soils seem to be overestimated.

The validity of the study Pillai et al. 1982 cannot be assessed based on the EU report. However, aniline was not found as a major degradation product in soil degradation study with aniline labelled desmedipham in two soils (Bruehl, R., 1978).

Study 4 – phenyl urethane

The adsorption behaviour of phenyl urethane (N-phenyl carbamic acid ethyl ester) was studied (**RAR B.8.1.3.1.2/04**, **2004**) in four soils in batch equilibrium. Phenyl urethane was applied at concentrations of 1.0, 0.5, 0.1, 0.05 and 0.01 mg/L in aqueous 0.01 M $CaCl_2$ solution. The study was performed in line with OECD test guideline 106 (2000). Parental mass balances were established for all soils and varied between 94 and 98 % of AR and in sterile soils (γ-irradiated) between 94 and 99 % of AR. Adsorption was found to be in the range of 28.8 to 45.9 % for soil A, 31.7 to 57.5 % for soil B, 35.2 to 46.3 % for soil C and 19.4 to 28.2 % for soil D. Freundlich adsorption coefficients (K_{Fads}) were in the range of 1.193 to 6.217 mL/g with corresponding adsorption constant $K_{OC(ads)}$ values ranging between 56.8 to 132.3 mL/g. The Freundlich exponent 1/n ranged from 0.802 to 0.950 (mean 0.861). The Freundlich exponents of the adsorption isotherms (1/n 0.802 to 0.950) indicated a slightly non-linear adsorption behaviour over a concentration range of two orders of magnitude (from 0.01 to 1 mg/L).

Volatilisation

Laboratory volatilisation studies

Based on the very low vapour pressure (1×10^{-8} Pa, 20 °C) obtained from laboratory volatility study (**RAR B.2.2/01**, **1990**) and low Henry´s law constant (5.4×10^{-7} Pa m^3 mol^{-1}) (**RAR B.2.2/02**, **2012**), desmedipham is virtually non-volatile. Therefore, significant exposure to air is not to be expected.

Volatilisation from soil and plant surface

Study 1 – desmedipham

The rate of volatilisation of ^{14}C-labelled desmedipham from bare soil and plant surface of Dwarf Bean (*Phaseolus vulgaris*) was studied (**RAR B.8.3.1/02**, **1994**). The study was performed according to the BBA, Part IV, 6-1. Soil and dwarf beans were sprayed with Kemifam D FL formulation (0.46 kg as/ha, considered as worst case application of desmedipham). The mean total recovery was 102 ± 11 % of AR and 97 ± 1.7 % of AR in soil and plant samples, respectively. Less than 20 % of the test substance evaporated in both experiments within 24 hours under the test conditions.

Study 2 – desmedipham

Studying the rate of volatilisation of ^{14}C-labelled desmedipham from bare soil and plant surface Dwarf Bean (*Phaseolus vulgaris*) was continued in the second study. The second study (**RAR B.8.3.1/03**, **1995**) was performed according to the BBA, Part IV, 6-1. The soil and dwarf beans were dosed with 0.98 kg as/ha using Kemifam D FL formulation. The mean total recovery was 91.2 ± 9.3 % of AR and 99.2 ± 4.0 % of AR in soil and plant samples, respectively. Less than 20 % of the test substance evaporated in both experiments within 24 hours under the test conditions. Compared with the previous study, the higher application amount and lower relative humidity did not increase the volatility of desmedipham.

Volatilisation from plant surface

Study 3 – desmedipham

The rate of volatilisation of desmedipham applied to Dwarf Bean (*Phaseolus vulgaris*) was determined (**RAR B.8.3.1/04**, **2005**) for the test item desmedipham/ethofumesate/phenmedipham 21/128/62 g/L EC was studied. The study was performed according to the BBA, Part IV, 6-1. Application rate of desmedipham was 0.125 kg/ha with water amount of 400 L/ha. The study was performed according to the BBA, Part IV, 6-1.

The volatilisation of ^{14}C desmedipham after spray application of desmedipham/ethofumesate/ phenmedipham 21/128/62 g/L EC from plant leaves was determined to be 9 % after 24 h. Thus, volatilisation effects are considered to be negligible under test conditions. Compared with the previous studies, the lower application amount did not increase the volatility of desmedipham.

11.4 Bioaccumulation

A brief summary of relevant studies on bioaccumulation, listed in the Renewal Assessment Report (RAR), is reported below. Only the information considered adequate, reliable and relevant for the classification proposal of desmedipham has been included.

Table 42: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Measured partition coefficient and bioaccumulation test data			
OECD TG 107: Partition Coefficient (n-octanol/water): Shake Flask Method (1995) Desmedipham technical (chemical purity 99.6 %) GLP compliant	**N-octanol/water partition coefficient of Desmedipham:** log P_{ow} = **3.5** at 23 °C	The partition coefficient was determined at pH 4 only due to the lack of stability above pH 7.	2016 RAR B.2.7/01 M-557369-01-1
OECD TG 117: Partition Coefficient (n-octanol/water): HLPC method (1995) EHPC technical (chemical purity 99.0 %) GLP compliant	**N-octanol/water partition coefficient of EHPC (AE F132319):** log P_{ow} = **0.87** at 40 °C P_{ow} = 7.48 at 40 °C	Acceptable.	2004 RAR B.2.7/01 M-233831-01-1
OECD TG 107: Partition Coefficient (n-octanol/water): Shake Flask Method (1995) Aniline technical (chemical purity ≥ 99.5 %) Non GLP	**N-octanol/water partition coefficient of aniline (M15):** log P_{ow}= **0.9** at 20 °C	Acceptable. The partition coefficient of the M15 (aniline) cited in the AIR dossier is the one considered in the European Union risk assessment report of aniline.	2004 RAR B.2.7/01 M-492497-01-1
OECD TG 117: Partition Coefficient (n-octanol/water): HLPC method (1995) Phenol technical (chemical purity ≥ 99.8 %) Non GLP	**N-octanol/water partition coefficient of phenol (M16):** log P_{ow} = **1.47** at 20 °C	Acceptable.	2006 RAR B.2.7/01 M-491666-01-1
OECD TG 117: Partition Coefficient (n-octanol/water): HLPC method (1995)	**N-octanol/water partition coefficient of diphenyl urea (AE F132317, M17):** log P_{ow} = **2.3** at 25 °C	Acceptable. The analysis was conducted at pH 5, 7 and 9.	2014 RAR B.2.7/01 M-485127-01-1

Method	Results	Remarks	Reference
Diphenyl urea technical (chemical purity 99.8 %) GLP compliant	P_{ow} = 200 at 25 °		
US EPA-FIFRA: OPP 165–4: Laboratory Studies of Pesticide Accumulation in Fish(1982) [aniline-UL-^{14}C]-desmedipham (specific activity: 3.18 MBq/mg and radiochemical purity 98.0 %) GLP compliant	The whole fish **BCF value** of 78 L/kg was calculated by the BIO-FAC model in **Lepomis macrochirus** (bluegill sunfish) at 0.06 mg/L concentration.	Mean recovery data for ^{14}C-desmedipham in tissue sample oxidations were 99 % for fillet, 98 % for whole fish, and 98 % for viscera (^{14}C-Benzoic acid was used to determine oxidizer efficiency). However, there was no attempt to characterise the radioactivity in water and the metabolites and degradation products were analysed only in fish tissues. Therefore, the **results** mainly **represent** the **bioaccumulation potential** of the **two degradates**, EHPC (M2) and N-(3-hydroxyphenyl)-acetamide (M3), and it is not possible to reassess the BCF of desmedipham from this study.	1993 & 2017 RAR B.9.2.2.3/01 & RAR B.9.2.2.3/03 M-146924-01-1 & M-578715-01-1
OECD TG 305E: Bioaccumulation: Flow-through Fish Test (1981) [phenoxy-UL-^{14}C]-desmedipham (specific activity: 50.17 µCi/mg and radiochemical purity ≥ 97 %) GLP compliant	Original desmedipham **BCF values** in ***Oncorhynchus mykiss*** (rainbow trout) at **low conc. (6.2 µg/L):** 157.3 L/kg **high conc. (62 µg/L):** 147.7 L/kg Reassessed desmedipham **BCF values** in **low concentration:** 333.9 L/kg **high concentration:** 317.7 L/kg	The mean measured **total radioactive recovery** in test water was near nominal (98.9-104 %). The BCF factor was calculated from the concentration of radioactivity in the fish or fish parts at plateau level (C_f) related to the average concentration of parent equivalents in water (C_w) during exposure using the formula: **BCF = C_f / C_w**. Reassessed BCF values were based on the lowest measured concentrations of DMP (47.1 and 46.5 % of AR for the low and the high concentration, respectively) of in water. The new BCF values, reflecting full bioconcentration potential of DMP, were calculated by dividing the original BCF values with 0.471 and 0.465 for the low and the high concentration, respectively.	1994 & 2017 RAR B.9.2.2.3/02 & RAR B.9.2.2.3/03 M-147020-01-1 & M-578715-01-1 **Key study**
OECD TG 305: Bioconcentration: Flow-through Fish Test (1996) [aniline-UL-^{14}C]-desmedipham (specific activity:	Desmedipham **BCFss values** in ***Oncorhynchus mykiss*** (rainbow trout) at **low conc. (100 µg/L):** 64 L/kg **high conc. (500 µg/L):** 65 L/kg	The uptake rate constant (k1), the depuration rate constant (k2), the resulting BCF and the **steady state bioconcentration factor** (BCFss) were calculated using data for the parent compound in whole fish.	2004 RAR B.9.2.2.3/03 M-587443-01-1 **Key study**

Method	Results	Remarks	Reference
53 mCi/mmol and radiochemical purity > 99.5 %) GLP compliant			

11.4.1 Estimated bioaccumulation

No studies available.

11.4.2 Measured partition coefficient and bioaccumulation test data

Three bioaccumulation studies and one re-evaluation report were included in the RAR. The studies were conducted generally in line with the OECD test guideline 305 "Bioaccumulation in Fish: Aqueous and Dietary Exposure" (2012). One of the studies was considered valid in the RAR for the determination of bioaccumulation and two were used as supporting data. Studies resulted in maximum steady state bioconcentration factors (BCF_{SS}) of **64** and **333.9** L/kg in whole fish (no growth correction or lipid normalisation applied) at concentrations of **100** and **6.4** µg/l, respectively. Experimentally derived BCF values are preferred for classification purposes of bioaccumulation.

The log P_{OW} for desmedipham and its degradates partition coefficients were estimated by conducting tests according to OECD test guidelines 107 and 117 (**RAR B.2.7/01, 2004-2016**). The study considered valid on partition coefficient n-octanol/water of **desmedipham** resulted in log P_{OW} value of 3.5. Desmedipham degradation products **EHPC** (log P_{OW} = 0.87), **aniline** (log P_{OW} = 0.9), **phenol** (log P_{OW} = 1.47) and **diphenyl urea** (log P_{OW} = 2.3) are not considered as bioaccumulative as their log P_{OW} does not exceed 3. Experimentally derived partition coefficients are considered as supportive type of data for classification purposes of bioaccumulation.

The endpoints are presented in table (Table 42) above and the studies are summarized below.

Study 1 – desmedipham

In the first study (**RAR B.9.2.2.3/01**, **1993** & **B.9.2.2.3/04**, **2017**), the bioaccumulation of desmedipham was tested with 240 fish (Lepomis macrochirus; bluegill sunfish) exposed for 10 days at test concentration of 0,6 mg/L followed by a 7-days depuration period. The study was conducted according test guideline OPP 165–4: Laboratory Studies of Pesticide Accumulation in Fish (1982). However, also the validity criteria for the updated OECD test guideline 305 (2012) were met: the water temperature variation was less than ± 2 °C, the concentration of dissolved oxygen did not fall below 60 % saturation and no mortality was reported in any test group or control. The concentrations of the test solutions as parent equivalents were 0.056 ± 0.0022 mg equivalents/L (average 93 % of nominal 0.06 mg/kg). Mean recovery data for ^{14}C-desmedipham in tissue sample oxidations were 99 % for fillet, 98 % for whole fish, and 98 % for viscera (^{14}C-Benzoic acid was used to determine oxidizer efficiency).

Uptake plateau phase was achieved by day 4. Tissue concentrations were 1.1 mg/kg in edibles, 8.9 mg/kg in non-edibles and 5.6 mg/kg in whole fish after 10 days. These values correspond to day 10 bioconcentration factors of 20 x, 77 x and 159 x for the fillet, whole fish and viscera, respectively. Daily BCF values for the uptake phase of the study ranged from 2.1 to 20 for fillet, 6.3 to 98 for whole fish, and 10 to 159 for viscera. Radio analysis of fish throughout the depuration period indicated 90, 91, and 93 % depuration from fillet, whole fish, and viscera, respectively, within 7 days.

This method estimated the uptake rate constant (k_1) mg/kg fish/mg/L water/day of 48 ± 6.4, first day depuration rate constant (k_2) of 0.62 ± 0.08, time for 50 % depuration of 1.1 ± 0.14 days, bioconcentration

factor (BCF) of 78 ± 14 and a time to reach 90 % of steady state 3.7 ± 0.48 days. The BIOFAC calculated BCF value was 96 % of the observed mean whole fish bioconcentration factor of 81 x for days 4, 7 and 10.

In the degradation product study, most of the residues were extractable and could be identified before and after hydrolysis with β-glucuronidase. A small amount of parent compound was recovered in a conjugated form. The principal tissue residues were identified as EHPC (M2) and N-(3-hydroxyphenyl)-acetamide (M3), which were present both in free and conjugated, possibly as glucuronides.

Since the active substance was almost totally hydrolysed in the study and the results mainly represent the bioaccumulation potential of the two degradation products, the BCF value could not be calculated based on desmedipham only. However, an attempt to recalculate the BCF on the concentration of DMP only was made but as radioactivity in water was not characterised in the study and the metabolites and degradation products were analysed only in fish tissues, it was impossible to re-assess the BCF of desmedipham from this study. Therefore, the study is considered as supporting information only.

Study 2 – desmedipham

In the second study (**RAR 8.2.2.3/02**, **1994** & **B.9.2.2.3/04**, **1993**), the bioaccumulation of desmedipham was tested with 75 fish in 6.2 µ/L group, 73 fish in 62 µg/L group and 25 fish in control group. The fish (*Oncorhynchus mykiss*; Rainbow trout) were exposed for 7 days in a flow-through system followed by a 14-day depuration period. Temperature was 15.3-17.0 °C, pH 7.7-8.1 and oxygen concentration 7.0 to 9.2 mg/L. Fish samples were taken after 0.4, 1.2, 2.3, 5 and 7 days of exposure (6 fish each time) and after 1, 3, 7, 10 and 14 days of depuration (3-6 fish each time). Whole fish were analysed for total activity by LSC. Non-edible and edible parts were analysed by TLC. Water samples were analysed for total ^{14}C-activity by LSC and for the parent by TLC. The study was generally in line with the OECD test guideline 305E (1981). However, also the validity criteria for the updated OECD test guideline 305 (2012) were met: the water temperature variation was less than ± 2 °C, the concentration of dissolved oxygen did not fall below 60 % saturation and no mortality was reported in any test group or control.

At the low dose level, plateau levels in edibles, non-edibles as well as whole fish were reached within 5 days amounting to plateau values of 0.292, 1.878 and 1.016 µg parent equivalents/g, respectively. Thereafter, radioactivity was depurated from fish and fish parts during 14 days with half-lives of 10.6 to 11.5 hours (non-edibles, whole fish) and 3.9 days (edibles). At the end of the depuration period (14 days), concentrations ranged from 0.057 to 0.095 µg/g. At the high dose, plateau levels for edibles, non-edibles and whole fish were reached within 5 days, amounting to plateau values of 2.320, 13.989 and 9.060 ug parent equivalents/g, respectively. Thereafter, as compared to the low dose, about the same depuration half-lives of 10.9 to 24.5 hours for non-edibles and whole fish as well as 2.9 days for edibles were obtained. Concentrations decreased to 0.360-0.588 ug/g at the end of the depuration period.

The mean measured total radioactive recovery in test water was near nominal (98.9-104 %). Desmedipham concentrations varied between 47.1 and 78.3 % in the lower dose level and between 46.5 and 79.9 % in the higher dose level. Besides desmedipham, at least four degradation products were detected of which bisphenylurea was tentatively identified. BCF values calculated in fish as a whole were 157.3 and 147.7 at concentrations of 6.2 and 62 µg/L, respectively.

Since the active substance concentrations were within 46-80 % of nominal, the results represent the bioaccumulation potential of desmedipham and its degradation products. However, the study has been re-evaluated to calculate the BCF on the concentration of DMP only. DMP accounted for 47.1 to 78.3 % of AR in the low exposure concentration and for 46.5 to 79.9 % of AR in the high exposure concentration. In order to reflect the bioconcentration potential of DMP more accurately, the BCF was based on the lowest measured concentration of DMP in water.

The recalculated BCF values are considered worst-case values for DMP due to the fact that they are based on the lowest measured concentrations of the parent substance in water and, secondly, an unknown amount of the residues measured in fish may be the result of the bioconcentration of the degradation products formed in water. Based on this conservative approach, i.e. minimizing the concentration in water and maximising the concentration in fish, results yield worst-case BCF values at steady state in the range of 317.7 – 333.9 L/kg in whole fish.

Study 3 – desmedipham

In the third study (**RAR B.9.2.2.3/03**, **2004**), the bioaccumulation of desmedipham was tested with 85 fish (*Oncorhynchus mykiss*; rainbow trout) at nominal test concentrations of 100 and 500 µg/L. The control group were exposed for 3 days in a flow-through system followed by a 5-6-day depuration period. Temperature was 13.2-15.2 °C, pH 6.3-7.1 and oxygen saturation > 60 %. Fish samples were taken after 0, 1, 2, 4, 14, 26, 36 and 48 hours of exposure (4 fish each time; except 6 fish at 48 hours). Because four fish became affected (thumbling) at nominally 500 µg/L during the third day, the uptake phase was stopped earlier for the test concentration and the affected fish excluded from the evaluation. After 72 hours, 55 fish were transferred to new aquaria for depuration phase. 4 to 6 fish were sampled during depuration phase at each time. All samples were analysed for total radioactivity and desmedipham concentrations by HPLC.

The study was generally in line with the old OECD test guideline 305 (1996). However, some of the validity criteria for the updated OECD test guideline 305 (2012) were met as well: the water temperature variation was less than ± 2 °C, the concentration of dissolved oxygen did not fall below 60 % saturation. However, 6 of 85 fish (7 %) were dead in the control vessel and 18 fish (21 %) at 100 µg/L were dead. Because there were absolutely no signs of intoxication of fish in both vessels until the evening of the fourth day (82 hours), it is assumed that a temporary dosing problem of phosphoric acid or dilution water in the night was responsible for the mortality. However, the pH measurement after 96 hours did not indicate a dosing problem (any longer). In spite of the low pH, test item concentrations in water remained constant only for 48 hours at mean measured concentrations (range) of 110 (100-125) µg/L and 315 (288-384) µg/L. After 48 hours, radioactivity in the water indicated constant dosing, but desmedipham was obviously degraded. Since according to the OECD test guideline 305 valid results can only be obtained with stable substances, bioconcentration can only be determined when based on the first 48 hours of the test and the depuration phase.

The concentrations of the active substance were within ± 20 % of nominal and the results were calculated over the period where the validity criteria were met. Hence the results represent the bioaccumulation potential of desmedipham. The uptake rate constant (k_1), the depuration rate constant (k_2), the resulting BCF and the steady state bioconcentration factor (BCF_{SS}) were calculated using data for the parent compound in whole fish. In this study, it was assumed that the steady state was reached if three successive Cf values varied by less than 20 %. Maximum and minimum values of the three last measurements before and including 48 hours varied by less the 20 % of the maximum values, which is in correspondence to the definition of the steady state in the OECD test guideline 305. Thus, the BCF_{SS} was calculated by dividing the mean of the 26, 36, and 48 h values for desmedipham in fish (µg/kg) by the mean of the corresponding concentrations in the water (µg/L). Results were similar at both nominal concentrations of 100 and 500 µg/L, ie. resulting BCF_{SS} values 64 and 65 L/kg, respectively. In order to assess total bioconcentration of degradation products, BCF_{SS} value of 72 was calculated for the total radioactivity in fish.

11.4.3 Conclusion on bioaccumulation

The log K_{OW} value for **desmedipham** (3.5) and for the degradation products **aniline** (0.9), **EHPC** (0.87), **phenyl** (1.47) and **diphenyl urea** (2.3) were measured according to OECD test guidelines 107 and 117. In the experimental studies according to the OECD test guideline 305 “Bioconcentration: Flow-through Fish Test” (1996), the highest BCF_{SS} values in whole fish for **desmedipham only** was 65 L/kg and for **desmedipham and the degradates** was 333.9 L/kg (as determined from ^{14}C-labelled compounds).

11.5 Acute aquatic hazard

Evaluation of acute aquatic hazard for desmedipham is based on studies which are considered valid in the Renewal Assessment Report (2017) of desmedipham (RAR annexed to this CLH proposal). All valid studies are presented in the table below and relevant studies for the classification purpose are also summarised below. More details can be found in the annexed RAR.

Table 43: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
Acute toxicity to fish - Desmedipham					
OECD 203; Static-renewal (daily) 96 h GLP	*Cyprinus carpio* (common carp)	Desmedipham technical Purity 98.2 % w/w	LC_{50} 4.83 mg/L with a 95 % confidence interval from 1.96-11.9 mg a.s./L (based on geometric mean measured concentrations of desmedipham) Endpoint mortality, LC_{50}	Deviation from the guideline not specified Fulfilled the validity criteria OECD 203 (1992)	2004, 2017 M-232623-01-1 M-594667-01-1 RAR B.9.2.1/06
OECD 203 Static-renewal (daily) 96 h GLP	*Oncorhynchus mykiss* (rainbow trout)	Desmedipham technical Purity 98.9 % w/w	**LC_{50} 1.41 mg a.s./L** (based on geometric mean measured concentrations of desmedipham) Endpoint mortality, LC_{50} **Key study**	Deviation not specified Fulfilled the validity criteria OECD 203 (1992)	2016 M-564890-01-1 RAR B.9.2.1/14
Acute toxicity to fish – degradate EHPC					
OECD 203; US EPA OCSPP: 850.1075 Static-renewal (daily) 96 h GLP	*Oncorhynchus mykiss* (rainbow trout)	EHPC (ethyl-3-hydroxyphenylcarbamate Purity 97.7 % w/w	LC_{50} 42 mg/l (based on nominal concentrations as measured EHPC concentrations were within 20 % of nominal) Endpoint mortality, LC_{50}	Deviation from the guideline not specified. Fullfilled the validity criteria OECD TG 203 (1992)	2000 M-197805-01-1 RAR B.9.2.1/07
OECD 203 (1992) Static 96 h GLP	*Oncorhynchus mykiss* (rainbow trout)	EHPC (ethyl-3-hydroxyphenylcarbamate Purity > 99 % w/w	LC_{50} 44 mg/l (based on nominal concentrations as measured EHPC concentrations were within 20 % of nominal) Endpoint mortality, LC_{50}	Deviation from the guideline not specified. Fulfilled the validity criteria OECD TG 203 (1992)	1998 M-494068-01-1 RAR B.9.2.1/09
OECD 203	*Danio rerio*	EHPC (ethyl-3-hydroxyphenylcarbamate	LC_{50} 113 mg/l (based on nominal	Deviation from the guideline not	2003 M-494065-

Static 96 h GLP	(zebra fish)	Purity 95.7 – 98.8 % w/w	concentrations as measured EHPC concentrations were within 20 % of nominal) Endpoint mortality, LC_{50}	specified. Fulfilled the validity criteria OECD TG 203 (1992)	01-1 RAR B.9.2.1/10
FIFRA 72-1, OPPTS 850.1075, OECD 203 Static 96 h GLP	*Pimephales promelas* (fathead minnow)	EHPC (ethyl-3-hydroxyphenylcarbamate Purity 99.4 % w/w	LC_{50} 73.1 mg/l (based on mean measured concentrations) Endpoint mortality, LC_{50}	Guidelines were harmonized for various test parameters (i.e. temperature, light, etc.) to achieve optimal environmental conditions for the test organisms. Fulfilled the validity criteria OECD TG 203 (1992)	2013 M-460190-02-1 RAR B.9.2.1/11
Acute toxicity to fish – degradate Aniline					
No information on guideline or GLP Flow-through 96 h	*Oncorhynchus mykiss* (rainbow trout)	Aniline No information on the purity	LC_{50} 10.6 mg/l (based on nominal concentrations as the mean concentration of aniline stayed within 20 % of nominal) Endpoint mortality, LC_{50}	Concentrations of aniline were within 20 % of nominal, Rainbow trouts were smaller (2.9 ± 0.3 cm) than recommended by the guideline (5.0 ± 1.0 cm). Fulfilled the validity criteria set in the OECD 203 (1992).	1982 M-255853-01-1 RAR B.9.2.1/12
Acute toxicity to fish – degradate Phenol					
No information on guideline or GLP 96 h	*Oncorhynchus mykiss* (rainbow trout)	Phenol No information on the purity	LC_{50} 5 mg/l (based on nominal concentrations, semi-static test, without measured test compound) LC_{50} 8.9 mg/l (based on measured concentrations, a flow-through system)	Test result available for nine fish species with different test systems regimes. The lowest LC_{50} value provided here. The tests were evaluated and considered	2003 M-491668-01-1 RAR B.9.2.1/13

			Endpoint mortality, LC_{50}	valid under Council Regulation (EEC) 793/93, European RAR	
Acute toxicity to fish – degradate Diphenyl urea					
No study was performed due to animal welfare reasons. However, the acute hazard assessment for fish is passed assuming that the metabolite has the same toxicity as the parent.					
Acute toxicity to *Daphnia magna* - Desmedipham					
OECD 202; USEPA 72-2; US EPA OCSOO: 850.1010; not specified Flow-through 48 h GLP	*Daphnia magna* (cladoceran)	Desmedipham technical Purity 96.8 % w/w	EC_{50} 0.78 mg/l (nom) **0.35 mg/**l (based on atrithmetic mean measured concentrations) Endpoint immobilisation, EC_{50} **Key study**	Fulfilled the validity criteria OECD TG 202.	1996, 2016 M-146483-01-1 M-545523-01-1 RAR B.9.2.4.1/03
OECD 202; U.S. EPA OPPTS Nr. 850.1010, deviation not specified Flow-through 48 h GLP	*Daphnia magna* (cladoceran)	Desmedipham technical Purity 99.5 % w/w	EC_{50} > 1.1 mg/l (based on mean measured concentrations of sum of parent and metabolite) EC_{50} > 0.33 mg/l (based on arithmetic mean measured concentrations of desmedipham) Endpoint immobilisation, EC_{50}	Fulfilled the validity criteria OECD TG 202.	2012 M-438144-02-1 RAR B.9.2.4.1/05
Acute toxicity to *Daphnia magna* - degradate EHPC					
OECD 202; US EPA OPPTS 850.1010 not specified Static 48 h GLP	*Daphnia magna* (cladoceran)	EHPC (ethyl-3-hydroxyphenylcarbamate Purity 97.7 % w/w	EC_{50} 12 mg/l (based on nominal concentrations as measured EHPC concentrations stayed within 20 % of nominal) Endpoint immobilisation, EC_{50}	Fulfilled the validity criteria OECD TG 202	2000 M-197806-0 RAR B.9.2-4-1/06
ISO International Standard 6341, the EEC directive 92/69, Part C.2.; OECD	*Daphnia magna* (cladoceran)	EHPC (ethyl-3-hydroxyphenylcarbamate Purity > 99 % w/w	EC_{50} 22 mg/l (based on nominal concentrations as measured EHPC concentrations stayed within 20 % of nominal) immobility	Fulfilled the validity criteria OECD TG 202	1998 M-494070-01-1 RAR B.9.2.4.1/07

202; not specified Static 48 h			Endpoint immobilisation, EC_{50}		
Acute toxicity to *Daphnia magna* – degradate Aniline					
Static 48 h	*Daphnia pulex* (cladoceran)	Aniline Purity > 98 %	EC_{50} 0.1 mg/l Endpoint immobilisation, EC_{50}	No information whether the study fulfils the validity criteria set in the OECD TG 202 and whether the concentrations were analysed from the test medium.	2002, 1983 M-240880-01-1 M-253903-01-1 RAR B.9.2.4.1/08
Acute toxicity to *Ceriodaphnia dubia*– degradate Phenol					
48 h No information on the guideline or GLP	*Ceriodaphnia dubia* (cladoceran)	Phenol Purity no information	EC_{50} 3.1 mg/l Endpoint immobilisation, EC_{50}	Validity criteria were not discussed. The report states that the study was reliable. Results based on measured concentration (not presented) European RAR 2006 under regulation (EEC) 793/93	2006 M-491666-01-1 RAR B.9.2.4.1/09
Acute toxicity to *Daphnia magna* - degradate Diphenyl urea					
EU Directive 91/414/EEC; Regulation 1107/2009 (Europe); US EPA OCSPP 850.1010; none Static 48 h GLP	*Daphnia magna* (cladoceran)	N,N'-diphenylurea (BCS-AA12039) Purity 99.8 % w/w	EC_{50} > 1 mg/l (based on nominal concentrations as measured concentrations stayed within 20 % of nominal) Endpoint immobilisation, EC_{50}	Fulfilled the validity criteria OECD TG 202	2014 M-501327-01-1 RAR B.9.2.4.1/09
Acute toxicity to *Americamysis bahia* - Desmedipham					
OPTTS Guideline	*Americamysis bahia* (mysid	Desmedipham technical	LC_{50} 1.2 mg/l a.s./L	Periodic analyses of	2011

850.1035; Flow-through 96 h GLP	shrimp)	Purity 99.5 % w/w	(based on mean measured concentrations of the sum of desmedipham and EHPC) LC_{50} 0.49 mg a.s./L (based on arithmetic mean measured concentrations) Endpoint mortality, LC_{50}	saltwater for potential contaminants were not conducted according with Good Laboratory Practices; how ever, these analyses were performed using a certified laboratory U.S.EPA analytical method	M-409869-01-1 RAR B.9.2.4.2/01
Acute toxicity to green algae - Desmedipham					
OECD TG 201 96 h static GLP	*Selenastrum capricornutum* *(green algae)*	Desmedipham technical Purity 96.8 % w/w	**RAR RMS: ErC50:** **~ 0.064 mg a.s./L** (estimated, based on geometric mean measured concentration) 24 h ErC50 0.059 mg/l 48 h E_rC_{50} 0.097 mg/l 72 h ErC50 0.228 mg/l (based on initially measured concentration of desmedipham) **Key study**	Desmedipham concentrations dropped dramatically towards the end of the study and were detected only in the two highest test concentrations on day 3. ErC_{50} has been calculated by the RMS during Peer review process of desmedipham. This approach was considered adequately acceptable during Peer Review Experts' meeting.	1993, 2004 M-146929-02-1 M-146929-02 RAR B.9.2.6.1/02
Acute toxicity to green algae – degradate EHPC					
OECD 201 Static 72 h GLP	*Pseudokirchneriella subcapitata* *(green algae)*	EHPC (Ethyl-3-hydroxyphenyl carbamate) Purity 97.7 % w/w	ErC_{50} 78 mg/L (based on nominal as measured concentrations stayed within 92-100% of nominal)	Study fulfilled validity criteria. pH range of the test medium was higher than recommended ±1.5 in OECD	2000, 2016 M-197998-01-1 M-197998-01-1 RAR B.9.2.6.1/06

			Endpoint growth rate: E_rC_{50}	TG 201 (2011)	
OECD TG No. 201, Adopted June 7; deviation not specified 72 h GLP	*Pseudokirchneriella subcabitata* (green algae)	EHPC (ethyl-3-hydroxyphenylcarbamate Purity > 99 % w/w	ErC_{50} > 60 mg/l (based on nominal concentrations) Endpoint growth rate, ErC_{50}	Study fulfilled the validity criteria for control growth and CV for section-by-section specific growth rate and average specific growth rates during the whole tes period in control OECD TG 201 (2011)	1998, 2016 M-494076-01-1 M-545521-01-1 RAR B.9.2.6.1/07
Acute toxicity to green algae – degradate Aniline					
Guideline not stated No GLP	*Pseudokirchneriella subcapitata* *(green algae) Selenastrum capricornutum* (green algae)	Aniline No information on purity	EC_{50} 19 mg/l (based on nominal concentations)	Does not fulfill the validity criteria on the today's requirement. EU RAR	2002 M-240880-01-1 RAR B.9.2.6.1/08
Acute toxicity to green algae – degradate Phenol					
96 h No information on guideline or GLP	*Pseudokirchneriella subcapitata* (green algae)	Phenol No information on purity	EC_{50} 61.1 mg/l	The endpoint is obtained from EU evaluation of phenol as an existing substance carried out in the framework of Council Regulation (EEC) 793/93 (CSTEE, 2003)	2003 M-491668-01-1 RAR B.9.2.6.1/09
Acute toxicity to green algae – degradate Diphenyl urea					
OECD 201 Static 72 h GLP	*Pseudokirchneriella subcapitata (*formerly known as *Selenastrum capricornutum)* (green algae)	N,N'-diphenylurea (BCS-AA12039) Purity 99.8 % w/w	ErC_{50} > 1 mg/l (based on nominal concentrations) Endpoint growth rate, ErC_{50}	Fulfilled the validity criteria OECD 201 (2011)	2014 M-501586-01-1 RAR B.9.2.6.1/10
Toxicity to aquatic macrophytes – *Lemna gibba* - Desmedipham					
OECD 221; US EPA:	*Lemna gibba*	Desmedipham	ErC_{50} > 5.2 mg/l (based on initial	Validity criteria for	2002, 2016 M-241092-

123-2; deviation not specified Static-renewal (day 2 & 4) 7 d GLP	(duck weed)	Purity 98.2 % w/w	concentrations of desmedipham) ErC_{50} > 0.229 mg/l (based on geometric mean measured concentration of desmedipham) Endpoint frond number and weight,7d-ErC_{50}	control growth was met (OECD TG 221)	01-1 M-545827-01-1 RAR B.9.2.7/02
OECD 221, deviation not specified Static-renewal (3x/week) 14 d GLP	*Lemna minor* (duck weed)	Desmedipham technical Purity 97.4 ± 0.22 %	7d-ErC_{50} 0.85 mg/l (based on nominal concentration of desmedipham) 7d-ErC_{50} 0.40 mg/l (based on geometric mean measured concentration of desmedipham) Endpoint frond number and weight,7d-ErC_{50}	Fulfilled the validity criteria OECD TG 221	2004, 2016 M-494089-01-1 M-545827-01-1 RAR B.9.2.7/03
OECD 221 Static-renewal (3x/week) 7 d GLP	*Lemna gibba* (duck weed)	Desmedipham technical Purity 99.5 % w/w	7d- ErC50 8.41 mg/l (based on nominal concentrations of desmedipham) **7-d ErC_{50} 0.113 mg/l** (based on geometric mean measured concentrations**)** Endpoint frond area, 7d-ErC_{50} **Key study**	Fulfilled the validity criteria OECD TG 221 for control growth, pH did not increase more than 1.5 units, and the temperature was within 24± 2°C. Minor deviation in the documentation of the study, no impact on the test result.	2012, 2016, 2017 M-444430-01-1 M-545827-01-1 M-594283-01-1 RAR B.9.2.7/04
Toxicity to aquatic macrophytes – *Myriophyllum spicatum* - Desmedipham					
OECD 221, deviation not specified Static-renewal (days	*Myriophyllum spicatum* (Eurasian watermilfoil)	Desmedipham technical Purity 99.5 % w/w	14-d ErC50 > 5.0 mg/l (based on nominal concentrations of desmedipham) 14-d ErC50 > 0.05 mr/l (based on geometric mean measured concentrations)	Study was performed according to OECD 221, but followed in principle OECD 239 except the replication	2013, 2016 M-461454-01-1 M-545827-01-1 RAR B.9.2.7/08

4,6,8,11) 14 d GLP			Endpoint total shoot length, ErC50	was smaller than recommended. pH ranged more than required ±1.5. Criteria for control growth was met. Criteria set in OECD 239 were otherwise met.	
Toxicity to aquatic macrophytes – degradate EHPC					
FIFRA guideline 123-2[8], OCSPP guideline 850.440 [12] OECD 221 Static-renewal (day 3) 7 d GLP	*Lemna gibba* (duck weed)	EHPC Purity 99.8 % w/w	$E_rC_{50\ dry\ weight}$ 114 mg/l (based on nominal concentrations) $E_rC_{50\ frond\ \#}$ 125 mg/l (based on nominal concentrations)	Fulfilled the validity crieteria OECD TG 221 (2006) for control growth, pH did not increace more than 1.5 unit and temperature was within 24 ± 2°C	2013 M-468307-01-1 RAR B.9.2.7/05
Toxicity to aquatic macrophytes — degradate Aniline					
OECD 221 Static 7 d GLP	*Lemna gibba* (duck weed)	Aniline Purity 99.3 % w/w	$E_rC_{50\ frond\ \#}$ > 7.56 mg/l (based on mean measured concentrations) $E_rC_{50\ frond\ area}$ > 7.56 mg/l (based on mean measured concentrations)	A slight deviation of pH is explained and discussed with the preparation of the nutrient medium.	2013 M-452808-01-1 RAR B.9.2.7/06
Toxicity to aquatic macrophytes – degradate **Phenol**					
No information on guideline No information on GLP 7 d	*Lemna minor* (duck weed)	Phenol No information on purity	EC_{50} 171 mg/l	For phenol, the endpoints used for risk assessment are based on the EU evaluation of phenol as an existing substance carried out in the framework of Council Regulation (EEC) 793/93 (CSTEE, 2003; M-491668-01 and European	2006 M-491666-01-1 RAR B.9.2.7/07

				RAR, 2006).	

11.5.1 Acute (short-term) toxicity to fish

Two acute toxicity tests with desmedipham, four with metabolite EHPC, one with metabolite aniline and one with metabolite phenol on different fish species were considered valid in the RAR. For desmedipham, the lowest LC_{50} value of 1.41 mg/L was determined on *Oncorhynchus mykiss* (rainbow trout). For the metabolite EHPC toxicity values on *Oncorhynchus mykiss*, *Danio rerio* (zebra fish) and *Pimephales promelas* (fathead minnow) were ≥ 42 mg/l (LC50). For aniline LC_{50} value was determined to be 10.6 mg/l (*Onchohynchus mykiss*), and for phenol 8.9 mg/l. (*Onchohynchus mykiss*).

According to the CLP guidance, tests consistent with OECD test guideline 203 (or equivalent) should be used for classification. As the lowest determinded LC_{50} value for the test substances is higher than 1 mg/L, desmedipham or its metabolites are not considered hazardous for fish on short-term. All endpoints are presented in the table above and the most relevant studies (with desmedipham) are summarized below.

Study 1 - Desmedipham

2004, 2017. Acute toxicity of Desmedipham tech. to fish (*Cyprinus carpio*) (product code AE B038107 00 1D98 007). M-232623-01, M-594667-01-1, RAR B.9.2.1/06

Acute toxicity of desmedipham (purity 98.2 % w/w) to common carp (*Cyprinus carpio*) was studied in 96 h test which was conducted according to OECD 203 guideline and in compliance with GLP. Five test item groups with ten fish in each were exposed under static renewal condition to nominal concentrations of 0.625 mg/l, 1.25 mg/l, 2.50, 5.00 and 10.0 mg a.s/L. Analytical determinations were made in all freshly prepared test media as well as in aged test media at test initation and then daily up to the end of the exposure period revealing arithmetic mean measured test concentrations: 0.249, 0.629, 1.43, 2.85 and 11.9 mg a.s./L. In addition solvent control (dimethylformamide: 100 µL/L) and an untreated test water control was tested with 10 fish each, respectively. Sub-lethal and behavioural observations were made during the course of the study. Dissolved oxygen concnetrations ranged from 98 to 102 % oxygen saturation, the pH values from 7.2 to 7.3 and the water temperature from 21.7 to 22 °C in all aquaria over the whole testing period. The water hardness was between 40-60 mg CaCO2/L. Photoperiod was 16 hours light / 8 hours dark during test. Validity criteria of the study were met (mortality in controls < 10% and dissolved oxygen ≥ 80%). The 96 hour LC_{50} value was 5.38 mg/L based on arithmetic mean measured concentration. The 96 hour LC_{50} value was 4.83 mg/L with a 95 % confidence interval from 1.96 – 11.9 mg a.s./L based on geometric mean measured concentration of desmedipham.

Study 2 - Desmedipham

2016. Desmedipham tech. (BCS-AG74515); Acute toxicity to rainbow trout (Oncorhynchus mykiss) under semi-static conditions. M-564890-01-1, RAR B.9.2.1/14

Acute toxicity of desmedipham (purity 98.9 % w/w) to rainbow trout (*Onchorhynchus mykiss*) was studied during 96 h in a semi-static test (daily renewal) according to OECD 203 guideline and in compliance with GLP. Deviations from the guideline were not specified. Acclimation period (with less than 5% of mortality) before the test was at least 14 days, and 2 days before testing in the same conditions as during the test. There was one test vessel per concentration and each included 10 test organisms. Mean length of the fish was 3.8 ± 0.3 cm and mean body weight 0.5 ± 0.1 g. Nominal test concentrations were 0.250, 0.500, 1.00, 2.00, 4.00 mg a.s./L. Geometric mean measured concentrations were 0.233, 0.475, 0.999, 2.06 and 4.15 mg a.s./L. Control and solvent control (dimethylformamide at 0.1 µL/mL were included in the test. Nondissolved material was not observed during the test, the test media were clear and colorless. Temperature was 13.0-13.9 °C during the test and photoperiod was 16 hours light / 8 hours dark; light intensity was not specified. pH ranged 6.4-6.5 in freshly prepared medium, 6.7-7.1 in the aged medium. Due to the higher stability of the test item at lower pH, the test water pH was adjusted to approximately 6.5 before adding the test item. Water hardness was 40-60 mg $CaCO_3$/L and dissolved oxygen 96-103 % of saturation.

Fish were observed for mortalities and signs of intoxication of 4 h after the start of the exposure and then once a day. Dissolved oxygen, water temperature and pH values were determined daily in each aquarium.

Water temperature was additionally measured in the control aquarium. Test concentrations were measured in the test medium of all treatment levels and controls in all freshly prepared and aged test water samples. In the concentration of 2.00 and 4.00 mg desmedipham / L (nominal) only on day 0 new water samples were taken due to 100 % mortality after 4 h.

Lethal effects were observed in the two highest concentrations of 2.06 and 4.15 mg a.s./L as all fish were dead within 4 h. No further mortalities were observed during the test. No sub-lethal effects or abnormalities were observed in the controls and in concentrations 0.223 and 0.475 mg/L. At 0.999 mg a.s./L severe sub-lethal effects were observed in all fish after 4 h of exposure. At test termination (96 h) all fish of the 0.999 mg a.s./L test level showed sub-lethal effects such as laboured respiration, apathy, lying on their side or back and remained unusually long periods at the bottom of the aquarium.

The study fulfils the validity criteria set in the OECD TG 203 (1992); mortality in the controls < 10% and dissolved oxygen ≥ 60%. Since the concentrations were not within ± 20% of nominal concentrations the results are based on mean measured concentrations of desmedipham during the study period. The 96 hour LC_{50} value was 1.41 mg a.s./L based on geometric mean measured concentration of desmedipham.

Studies with the metabolite (EHPC, Aniline, Phenol)

All metabolites were acutely less toxic to fish than the parent substance desmedipham. Therefore, the studies with the metabolites presented in the Table 43 are not described here in detail (for more details see the annexed RAR of desmedipham).

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Two valid acute toxicity studies with desmedipham on *Daphnia magna* were available giving the lowest EC_{50} value of 0.35 mg/l. Also studies with metabolites EHPC (*Daphnia magna*), aniline (*Daphnia pulex*), phenol (*Ceriodaphnia dubia*), and diphenyl urea (*Daphnia magna*) were available with the toxicity range of 0.1 – 22 mg/l, although the study with aniline (EC_{50} 0.1 mg/l) is considerd a supportive study as there was no information on the validity criteria neither on the measurements of the test concentrations. There are, however, other study results available in the EU Risk Assessment Report on Aniline (2004) reporting results which were in line with the endpoint of this above mentioned aniline study. In addition, the toxicity of desmedipham to *Americamysis bahia* was also in the same range (LC_{50} 0.49 mg/l). Based on these test results desmedipham (or its metabolites) may be considered acutely toxic to aquatic invertebrates.

All endpoints are presented in the Table 43 and the most relevant studies are summarized below. Only studies with metabolites (Aniline, diphenyl urea) which were in same order of magnitude toxic to aquatic invertebrates than the parent substance desmedipham are presented here. Details for studies with EHPC and Phenol may be seen in the annexed RAR of desmedipham.

Study 1 - Desmedipham

1996, 2106. Desmedipham technical; 96.8 percent w:w – Daphnia acute toxicity. M-146483-01-1, M-545523-01-1 RAR B.9.2.4.1/03

Acute toxicity of desmedipham (purity 96.8 %) was studied in a 48 h flow-through test according the OECD 202; US EPA: 72-2 OCSOO: 850.1010 guidelines and in compliance with GLP. Twenty daphnids per concentration, divided into 2 groups of 10, were exposed to nominal concentrations of 0, 0 (solvent control), 0.41, 0.69, 1.2, 1.9 and 3.2 mg/L. The number of immobile daphnids per concentrations were recorded at 3, 6, 24 and 48 h. Desmedipham and EHPC were measured from test water at the beginning (0 h) and at the end of the test (48 h) by HPLC. LOD for both compounds were 0.1 µg/L. The arithmetic mean measured concentrations of desmedipham were 0, 0 (solvent control), 0.13, 0.32, 0.57, 0.93 nad 1.6 mg a.s./L. The pH of test was 7.4-7.5 (hardness 160-180 mg CaCO3/l). Test water temperature was within 20 ± 1 °C and oxygen saturation was over 90 %. A light cycle of 17 h light and 8 h dark was used.

The study fulfilled the validity criteria set in the OECD 202 guideline (mortality in the controls < 10% and dissolved oxygen ≥ 3 mg/L. The 48 hour EC_{50} value based on arithmetic mean measured concentration of desmedipham was 0.35 mg a.s./L (with 95 % confidence intervals of 0.28 – 0.42 mg a.s./L).

Study 2 - Desmedipham

2012. Desmedipham: a 48-h flow-through acute toxicity test with cladoceran (*Daphnia magna*) – Final report. M-438144-02-1, RAR B.9.2.4.1/05

Acute toxicity of desmedipham (purity 99.8 % w/w) to *Daphnia magna* was studied in a 48 hours flow-through test according to OECD 202; U.S. EPA OPPTS 850.1010 guidelines and in compliance with GLP. Twenty daphnids (≤ 24 h old) per concentration, 10 in each of two replicates, were exposed to desmedipham nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg a.s./L. A dilution water control and a solvent control (0.02 mL/L dimethylformamide) were included in the test. Observations to determine abnormal behaviour and immobility were made approximately 2, 24 and 48 hours after test initiation. Mean measured test concentrations were determined at 0, 3, 24 and 48 hours by HPLC-UV detection. Results were calculated based on both the combined desmedipham and EHPC mean measured test concentrations and on arithmetic mean measured concentrations of desmedipham. A photoperiod of 16 hours of light and 8 hours of darkness with light intensity at test initiation 738 lux at the surface of the water was used and temperature was 20 ± 1 °C and pH of the water ranged from 8.3 to 8.8.

The study fulfils the validity criteria set in the OECD TG 202; mortality in the controls < 10% and dissolved oxygen ≥ 3 mg/L. Since the metabolite EHPC is clearly less toxic than the parent the toxicity can be attributed to the active substance. Therefore the study can be considered valid and a 48-hour EC_{50} value of > 1.1 mg a.s./L was obtained based on mean measured test concentrations of sum of parent and metabolite. A 48-hour EC_{50} value of > 0.33 mg a.s./L was obtained based on arithmetic mean measured concentrations of desmedipham.

Study 3 – Aniline

1983, 2002. Comparison of the susceptibility of 22 freshwater species to 15 chemical compounds. I. (Sub)acute toxicity tests. M-240880-01-1, M-253903-01-1, RAR B.9.2.4.1/08

Daphnids less than 24 h old were exposed minimally in duplicate in standard reference water at 19ºC. Treatment groups consisted of 25 daphnids in 1-liter volume of treatment solution. Immobility of daphnids was determined after 48-h exposure.

The study was evaluated in the addendum 3 to DAR. The study was accepted then and the EC_{50} value was listed in the Review Report on Desmedipham (SANCO/4061/2001- final, 13 February 2004). The acute toxicity study with daphnids was performed in 1978. There is no information whether the study fulfils the validity criteria set in the OECD TG 202 and whether the concentrations were analysed from the test medium. There are, however, other study results available in the EU Risk Assessment Report on Aniline (2004) reporting a 48 h EC_{50} value of 0.16 mg/L (semi-static, measured concentrations, Danish EPA) and 0.25 mg/L (flow-through, measured concentration; multispecies test, Holcombe et al., 1987). These results are in line with the endpoint obtained above. Therefore, the lowest endpoint of 0.1 mg p.m./L was considered acceptable in RAR of desmedipham and also presented here.

Study 4 - Diphenyl urea

2014; Acute toxicity of BCS-AA12039 (N,N'-diphenylurea) to the waterflea Daphnia magna in a static laboratory test system -- Limit test -- Final report, M-501327-01. RAR B.9.2.4.1/09

Daphnia magna (1st instars < 24 h old) (10 replicates with 5 animals each per concentration; 50 animals per study group), were exposed in a static test system for 48 hours to an untreated control, a solvent control (Dimethylformamide; 100 µL DMF /L) and a limit concentration of 1.0 mg p.m./L1 without feeding. Immobility was assessed at 24 and 48 hours after starting the test. The content of metabolite in exposure media was measured in freshly prepared (immediately before distribution to the test vessel) and in aged test media from all replicates by HPLC-UV detection. Dissolved oxygen content ranged from 8.4 to 9.1 mg O2/L

and pH was 7.9. The temperature ranged from 18 - 22 °C (max deviation ± 1°C). The photoperiod was 16 hours of light and 8 hours dark.

The study fulfils the validity criteria set in the OECD TG 202; mortality in the controls < 10% and dissolved oxygen ≥ 3 mg/L. EC_{50} value was determined to be >1.0 mg p.m./L (95% confidence intervals not applicable).

Study 5 – Desmedipham (Americamysis bahia)

2011. Desmedipham: A 96-hour flow-through acute toxicity test with the saltwater mysid (*Americamysis bahia*) M-409869-01-1, RAR B.9.2.4.2/01

Acute toxicity of desmedipham (purity 99.5 %) to *Americamysis bahia* was studied in a flow-through test for 96 hours according to US EPA OPPTS Guideline 850.1035 and in compliance with GLP. Juvenile *Americamysis bahia* (< 24 h old) were exposed to six nominal test concentrations 0.063, 0.13, 0.25, 0.50, 1.0 and 2.0 mg desmedipham /L, respectively, to a negative control (dilution water) and a solvent control (0.1 mL/L acidified dimethylformamide). Two replicate test chambers were maintained in each treatment and control group, with 10 saltwater mysids in each test chamber, for a total of 20 mysids per test concentration. Observations of mortality and other signs of toxicity were made approximately 6, 24, 48, 72 and 96 hours after test initiation. Cumulative percent mortality observed in the treatment groups was used to determine LC_{50} values at 24, 48, 72 and 96 hours. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, at approximately 3.5 hours after initiation, at 24, 48, 72 hours and the end of the test by HPLC-UV. When measured concentrations of the samples collected during the test were averaged, the mean measured test concentrations for this study were 0.044, 0.13, 0.26, 0.53, 1.0 and 2.0 mg/L, representing 70, 100, 104, 106, 100 and 100% of nominal concentrations, respectively. Temperature during the study was 25 ± 2°C, pH 8.0-8.2 and dissolved oxygen 4.6-6.9 mg O2/L. The photoperiod was 16 hours of light and 8 hours of dark.

The study was considered to fulfill the validity criteria set in OPPTS Guideline 850.1035 (1996) for control mortality and dissolved oxygen. It was done in compliance with GLP, but periodic analyses of saltwater for potential contaminants were not conducted in accordance with Good Laboratory Practices; however, these analyses were performed using a certified laboratory and standard U.S. EPA analytical methods. According to the OPPTS Guideline a range finding test should be performed to determine which life stage, juvenile or young adults, is to be utilized in the definitive test which was not done. The 96-hour EC_{50} value is 1.2 mg/L based on mean measured concentrations of the sum of desmedipham and EHPC and and 0.49 mg a.s./L based on mean measured concentrations of desmedipham.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

During the Peer Review of phenmedipham the validity of available algae studies were discussed, since in most cases the analytical results showed initial residues, but at next sampling times the residues were below the LOQ. The approach of using endpoints based on geomean concentration of desmedipham chosen by RMS was not fully supported during the Peer Review Experts' meeting 133 if there are no intermediate measurements. Hence, studies with no intermediate samples with measurable residues were not considered valid. For the sake of consistency this approach has been taken also for desmedipham. Therefore, those studies which were not considered valid then are not presented in this CLH-dossier either, though, can be found only in the annexed RAR of desmedipham.

Only one toxicity study with desmedipham on green algae was considered valid in the RAR. In that study there was also severe problems with the stability of the test substance and the endpoint ErC_{50} has been estimated by the RMS (~0.064 mg/l a.s./L). This study has been used to derive aquatic acute classification for desmedipham. Also studies with aquatic macrophytes *Lemna gibba* (duck weed) and *Myriophyllum spicatum* (Eurasian watermilfoil) support the conlusion that desmedipham is acutely very toxic for aquatic plants.

Study 1 – Desmedipham

1993, 2004. Toxicity of Desmedipham technical to the green algae, *Selenastrum capricornutum*. M-146929-01-1, M146929-02, RAR B.9.2.6.1/02

The toxicity of desmedipham (purity 96.8%) to the green algae *Selenastrum capricornutum* was studied during 96 h in a static test according to OECD 201 guideline. Triplicate algal cultures with a cell count of approximately 1 x 10^4 cells/mL were exposed for 96 h to desmedipham technical in modified AAP algal medium at five nominal concentrations (i.e. 0.065, 0.11. 0.18, 0.3 and 0.5 mg/L). Six replicate control and solvent control cultures were also tested. Several preliminary trials were conducted prior to the definitive test in order to find an adequate method to maintain pH <7 but not to alter the nutrient content significantly. AAP growth medium was modified by reducing the concentration of NaHCO3 to 7.5 mg/L and increasing the concentration of K2HPO4 to 3.132 mg/L to reach pH of 6. Each flask was also purged with 0.5% CO2: air. The cell density of each culture was measured under a microscope, using a haemocytometer, at 24-hour intervals during the test.

The temperature range during the test was 23.5 - 23.8°C, with a mean temperature of 23.6°C. The pH was 6.0 in the beginning of the test and between 7.5 and 9.9 in the end of the test. Samples of each concentration were taken for analysis of desmedipham and EHPC by HPLC at test initiation (prior to addition of algae), every 24 hours (±1 hour), and at test termination (96 hours).

The measured concentrations of test substance at day 0 were used in all data calculations. Cell counts in the control and solvent control were significantly different at 48, 72, and 96 hours, and, therefore, the solvent control data was used for all calculations. The initial measured concentrations of desmedipham were: 0.053, 0.084, 0.141, 0.178, and 0.619 mg/L. Desmedipham was found on day 3 only in the two highest treatment levels in concentrations of 7 % (0.013 mg/L) and 3.2 % (0.020 mg/L) of initial, respectively (see **Table 44**). No desmedipham could be detected in any of the test treatments on day 4 due to degradation under test conditions, principally by hydrolysis and therefore results were based on initial measured concentrations. The 72 h ErC_{50} was 0.228 mg/L (95% confidence limits 0.168 - 3.08 mg/L), 48 h E_rC_{50} was 0.097 (95% confidence limits 0.085 – 0.115 mg/L) and 24 h E_rC_{50} was 0.059 mg/L (95% confidence limits 0.053 – 0.084 mg/L). The NOEC was less than 0.05 mg/L since the inhibition on cell growth was already identified in the lowest test concentrations of desmedipham (initial 0.053 mg/L).

Table 44 Results of the sample analyses

Nominal concentration Desmedipham mg/L	Day 0		Day1		Day 2		Day 3		Day 4	
	DMP mg/L	EHPC mg/L	DMP mg/L	EHPC mg/L	DMP mg/L	EHPC mg/L	DMP mg/L	EHPC mg/L	DMP mg/L	EHPC mg/L
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Solvent control	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
0.065	0.053	<LOD	LS	LS	<LOD	0.024	ND	<LOD	ND	0.024
0.108	0.084	0.011	<LOD	0.034	<LOD	0.027	<LOD	0.036	ND	0.055
0.18	0.141	0.019	0.021	0.071	0.013	0.075	ND	0.084	ND	0.087
0.30	0.178	0.023	0.041	0.120	0.064	0.083	0.013	0.134	ND	0.151
0.50	0.619	0.084	LS	LS	0.041	0.245	0.020	0.249	<LOD	0.259

ND= Not Detected, LOD=Limit of Determination (0.01 mg/L), LS=Lost sample, no result

The study fulfils the validity criteria for control growth (within 72 h). According to RAR mean coefficient of variation for section-by-section specific growth rates in control was < 35 %. CV of average specific growth rates during the whole test period in controls was obtained to be 1.7 (criteria < 7%). Since desmedipham has a hydrolysis half-life of 47-50 days, 19.6 hours, and 0.17 hours at pH values of 5, 7, and 9, respectively, modifications were made to the standard algal test medium to adjust the pH to 6 in order to prevent the hydrolysis of desmedipham. The low pH could not be maintained during the study due to CO_2 consumption by the algae and the pH increase during the study exceeded 3 units in the controls and the three lowest treatments. However, over the first 48 hours of the study, the pH in all replicates except solvent control remained within the range of 6.0 to 7.1. During the Peer review of desmedipham it was agreed that the change in pH is not a validity criteria, and this study was considered valid.

In the RAR an estimated 72 h E_rC_{50} value of 0.064 mg/L based on geometric mean measured concentration was calculated as following:

Desmedipham was found on day 3 only in the two highest treatment levels of 0.178 mg/L (nom 0.3 mg/L) and 0.619 mg/L (nom 0.5 mg/L) in concentrations of 7 % and 3.2 % of initial, respectively (see **Table 44**). The calculated 72 h E_rC_{50} value (based on initial measured concentration) was 0.228 mg/L which is close to the test concentration of 0.178 mg/L. The 72 h geomean measured concentration of 0.178 mg a.s./L was calculated to be 0.050 mg a.s./L. RMS has calculated the ratio between 0.228 mg a.s./L and 0.178 mg a.s./L which is 1.28. Hence, the estimated 72 h ErC50 would be 0.050 mg a.s./L * 1.28 = 0.064 mg/L. This value was used in the risk assessment.

In the test report it is suggested to use E_rC_{50} value either for 24 h 0.059 mg/L (95% confidence limits 0.053 to 0.084) or 48 h 0.097 mg/L (95% confidence limits 0.085 – 0.115 mg/L) as most relevant to a continuous desmedipham exposure. This was based on the observed substantial increase in cell growth after 48 hours of the test and when the pH began to increase, indicating recovery of the algal cultures as desmedipham degraded. The pH was maintained at ≤ 7.0 for at least 48 hours (increase < 1.5 units). Desmedipham degraded mainly due to hydrolysis, but it was argued in the test report that also absorption and/or metabolism by the algae, accelerated hydrolysis at the higher temperature used in the study (i.e. 24 °C), or photolysis due to the high light intensity (i.e. 8000 lux) might have affected together.

It is noted that the increase of growth followed the same pattern also in the controls without the exposure. Clear dose response can be seen in the results even though the test concentration dropped dramatically already during the first 24 h (see following figure). According to the test guidance if the deviation from nominal or initial concentration is not within the range of ± 20 %, as in this case with desmedipham, the results should be based on geometric mean concentration during exposure.

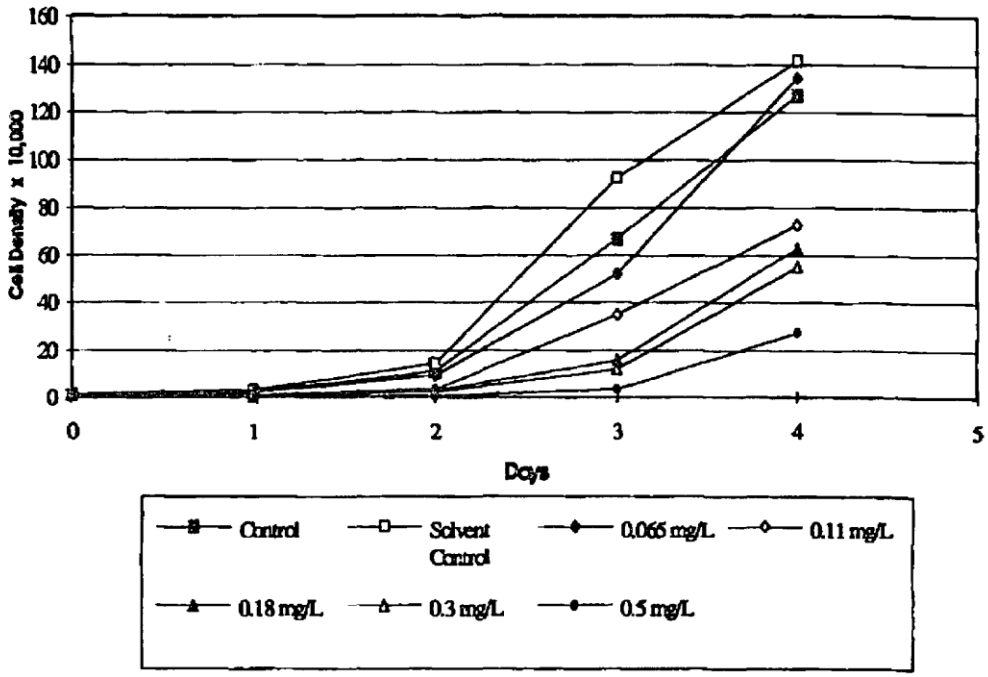


Figure 3 Algal Growth Curves

To support the approach accepted in Peer Review Process and estimated E_rC_{50} value of 0.064 mg/L the dossier submitter calculated the geomean concentrations using half of the limit of determination for those concentrations which were not detected on 72 h time point, resulting 0,016, 0,020, 0,027, 0,048, and 0,111 mg/L. In addition, inhibition per cents were recalculated for the period of 0 to 72 h and same results as presented in the test report were obtained (inhibition-% 13, 21, 39, 45, and 71, respectively). Plotting percentage of inhibition against geomean concentrations gives appr. same E_rC_{50} value with visual observation as used in the risk assessment (E_rC_{50} value of 0.064 mg/L). The validity of the test was also reconfirmed with calculations.

Dossier submitter suggests using estimated E_rC_{50} value of 0.064 mg/L also for classification purpose and as *Selenastrum capricornutum* was found to be the most sensitive species this result is used for derivation of aquatic acute classification for desmedipham. Approach using the 48 h E_rC_{50} value of 0.097 mg/L or 24 h E_rC_{50} value of 0.059 (based on the initial measured concentrations) would result the same classification.

Studies with the metabolites (EHPC, Aniline, Phenol, Diphenylurea)

All studies with metabolites revealed toxicity values > 1 mg, and basis of them the acute aquatic classification would not be warranted for desmedipham. Therefore, the studies with the metabolites on green algae presented in the Table 43 are not described here in detail (for more details see the annexed RAR of desmedipham).

Study 2 – Desmedipham (Lemna gibba)

2002, 2016. Effects to Lemna gibba (Duckweed) in a Growth Inhibition Test, Desmedipham, Technical, 98.2 %. M-545827-01-1, RAR B.9.2.7/02

The effects of desmedipham technical (purity 98.2 %) to duckweed *Lemna gibba* was studied during 7-days according to OECD 221 and US EPA guidelines. Three replicates of *Lemna gibba* were exposed to nominal concentrations of control, solvent control (0.1 ml dimethylformamide /L), 0.03, 0.09, 0.25, 0.72, 2.08 and 6.0 mg a.s./L for a 7-day period in a static-renewal system. 5 uniform healthy-looking plants (colonies) with 3

fronds each were used in each test vessel hence initial frond number was 15. Test solutions were renewed on days 2 and 4.

Test water was a 20X-AAP synthetic medium with a mean temperature of 24.7 °C and dissolved oxygen content of 7.2 mg/L. Light intensity ranged from 6500 to 7200 Lux. Chemical analysis of the freshly prepared (days 0, 2 and 4) and aged media (days 2, 4 and 7) was performed for all test concentrations by HPLC-UV detection. LOQ was 0.02 mg/L.

The method recovery was 92 %. The mean measured desmedipham concentration in the fresh test solution ranged from 71 to 86 % of nominal concentrations. Due to rapid hydrolysis, no residues were detected in the aged test solutions. The results were provided based on both mean measured initial concentrations and geometric mean measured concentrations of desmedipham.

Table 45 Geometric mean measured concentrations of desmedipham

Sampling time	**Measured concentrations (3 sampling intervals)**					
nom.conc. (mg/l)	**0.03**	**0.09**	**0.25**	**0.72**	**2.08**	**6**
0	0.019	0.0639	0.1584	0.5176	1.7341	5.1483
2	0.01	0.01	0.01	0.01	0.01	0.01
2	0.0224	0.0621	0.1733	0.4352	1.5241	4.934
4	0.01	0.01	0.01	0.01	0.01	0.01
4	0.0236	0.0755	0.2017	0.6165	1.8575	5.4802
7	0.01	0.01	0.01	0.01	0.01	0.01
geomean conc.	**0.0148**	**0.0261**	**0.0425**	**0.0729**	**0.1312**	**0.2286**

Highlighted cell measured value below LOQ (0.02 mg/L) so half the LOQ is used for calculations.

A t-test determined that control and solvent control results were not significantly different and therefore the results for controls were pooled.

Table 46 Growth inhibition in *Lemna gibba* after 7 days

Mean measured concentration [mg/L]	**Final frond no. (replicate means, day 7)**	**Inhibition of frond no. [%]**	**Specific Growth of frond no. rate***	**Inhibition of specific growth rate [%]**	**Frond dry weight**	**Inhibition dry weight [%]**
P. Control	863		0.481		0.0586	
0.02	921	-7	0.491	-2	0.0555	5
0.07	930	-8	0.489	-2	0.0573	2
0.18	895	-4	0.484	-1	0.0615	-5
0.52	831	4	0.472	2	0.0546	7
1.71	663*	23	0.440*	9	0.0468*	20
5.2	560*	35	0.410*	15	0.0385*	34

* Significantly different ($P < 0.05$) from pooled control group by Williams Test.

Both the 7 d E_rC_{50} and E_bC_{50} values were above 5.2 mg/L. Also, the 7 d NOEC values for growth rate, biomass and frond dry weight were 0.52 mg/L. The 7 d LOEC value was 1.71 mg/L.

The OECD TG 221 validity criteria for control growth were met. 7 d E_rC_{50} and E_bC_{50} values were >5.2 mg/L based on mean measured initial concentration of desmedipham and E_rC_{50} >0.229 mg a.s./L based on geometric mean measured concentration of desmedipham.

Study 3 – Desmedipham (Lemna minor)

2004, 2016. Desmedipham technical – Aquatic plant toxicity test Lemna minor, semi-static, 14d. M-49089-01-1, M-545827-01-1, RAR B.9.2.7/03

Toxicity of desmedipham technical (purity 97.4 ± 0.22 %) on *Lemna minor* was studied in a semi-static 14 d test (according to OECD 221 (Draft 2002)). *Lemna minor* was exposed in three replicates to 5 nominal concentration levels 0.032, 0.1, 0.32, 1.0 and 3.2 mg a.s./L and to a control. 3 uniform healthy-looking plants (colonies) with 4 fronds each were used in each test vessel hence initial frond number was 12. A semi-static test with renewal of test media every 2-3 days was performed and frond numbers were determined on start and end of the test and every renewal date of the test media. Therefore, frond numbers were assessed on days 0, 2, 5, 7, 9, 12 and 14 and inhibition of log biomass growth, specific growth rate and log biomass dry weight were determined.

The concentrations of the active ingredient desmedipham and its metabolite EHPC were analysed on days 0, 2, 9 and 12 (freshly prepared solutions) and on days 2, 5, 12 and 14 (old solutions) via HPLC. The recovery rates were calculated based on measured concentrations of desmedipham and the sum of the measured concentrations of desmedipham and EHPC.

The environmental conditions during the study were: water temperature of 24 ± 2 °C, a continuous fluorescent light regime 6500 – 10000 Lux on the surface of the test medium and pH-value of 7.0 ± 0.56. The pH was between 6.51 and 6.81 in the three highest test concentrations.

The recovery rates, calculated as a sum of the mean measured desmedipham and EHPC, were 92 ± 7 % in the fresh and 93 ± 8 % (n=20) in the old solution. RMS had requested that the endpoints should be calculated based on geometric mean measured concentrations of desmedipham and Task Force Desmedipham had provided following calculations:

Table 47 Geometric mean measured concentrations of desmedipham

Sampling time (d)	**Measured concentrations (2 sampling intervals = first 5 days covered)**				
nom conc (mg/l)	**0.032**	**0.1**	**0.32**	**1**	**3.2**
0	0.025	0.088	0.27	0.87	3.01
2	0.011	0.028	0.13	0.29	1.02
2	0.025	0.1	0.31	0.91	3.08
5	0.007	0.024	0.058	0.21	0.68
geomean conc.	**0.0145**	**0.0492**	**0.1533**	**0.4621**	**1.5623**

The study fulfilled the validity criteria for control growth and the pH-value was within the acceptable limits set in OECD TG 221 (2006). The original water temperature measurements were not presented, but the temperature was stated to be within 24 ± 2 °C. This missing information is not considered to invalidate the study since control growth fulfilled the validity criteria and hence the study is considered valid. The 7 day ErC_{50}-value was 0.85 mg a.s./L based on nominal concentrations of desmedipham and 0.50 mg a.s./L and 0.40 mg a.s./L based on arithmetic and geometric mean measured concentrations of desmedipham, respectively. The 7 day NOErC was 0.0492 mg a.s./L based on geomean measured concentrations of desmedipham.

Study 4 – Desmedipham (Lemna gibba)

2012, 2016, 2017. Lemna gibba G3 growth inhibition test with desmedipham (technical) under semi-static conditions. M-444430-01, M-545827-01-1, M-594283-01-1, RAR B.9.2.7/04

The effect of desmedipham technical (purity 99.5 % w/w) was studied under semi-static conditions according to OECD guideline 221. Three replicates of 12 fronds in each of *Lemna gibba* G3 per test concentration were exposed for 7 days to the nominal concentrations of 0.0780, 0.156, 0.313, 0.625, 1.25, 2.50, 5.00 and 10.0 mg a.s./L in comparison to a water control. Quantitative amounts of desmedipham and its metabolite EHPC were measured in all freshly prepared test levels on day 0, 2, and 5 and additionally in all aged test levels on day 2, 5, and 7 of the exposure period by HPLC-MS/MS. The pH values ranged from 7.5 to 7.8 and the incubation temperature ranged from 22.8°C to 24.6°C over the whole period of testing at a continuous illumination of 8267 Lux.

The analytical findings of the sum of desmedipham and EHPC in all freshly prepared test levels on days 0, 3, and 5 ranged between 110 and 125% of nominal concentrations (and between 101 and 125 % based on

desmedipham concentration). In aged test levels on days 3, 5, and 7, analytical findings ranged between 100 and 113% of nominal concentrations. All reported results are based on nominal values desmedipham. No desmedipham was detected in the aged medium.

Geometric mean measured concentrations are presented in the following table.

Table 48 Geometric mean measured concentrations of desmedipham

Sampling time (d)	**Measured concentrations (3 sampling intervals)**							
nom.conc. (mg/l)	**0.078**	**0.156**	**0.313**	**0.625**	**1.25**	**2.5**	**5**	**10**
0	0.0941	0.187	0.382	0.717	1.48	2.91	5.8	11
3	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006
3	0.0829	0.19	0.337	0.72	1.43	2.52	5.07	10.4
5	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.00649
5	0.0938	0.195	0.381	0.735	1.44	2.91	5.82	11
7	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.00836
geomean conc.	**0.0074**	**0.0107**	**0.0149**	**0.0208**	**0.0295**	**0.0409**	**0.0579**	**0.1650**

Highlighted cell measured value below LOQ (0.0012 mg/l) so half the LOQ is used for calclulations

There were no visual effects observed in any of the test concentrations. The effects of desmedipham on mean growth rate of frond number and of total frond area are presented in the following table.

Table 49 Effects of desmedipham to *Lemna gibba* (7-day growth inhibition test)

Nominal test concentration (mm) [mg/L]	**Final frond no. (replicate means, day 7)**	**Final total frond area of plants (replicate means) [mm^2]**	**% inhibition**	
			Mean growth rate for frond no.	**Mean growth rate for total frond area of plants**
control	205.0	1680.0	--	--
0.0780 (0.0074)	183.3	1450.0	3.8	2.0
0.156 (0.0107)	171.3	1298.0	6.6	5.6
0.313 (0.0149)	156.7	1199.3*	9.4	9.7
0.625 (0.0208)	130.7*	987.0*	15.8	16.1
1.25 (0.0295)	106.7*	778.0*	22.9	21.9
2.50 (0.0409)	85.0*	592.3*	31.2	29.8
5.00 (0.0579)	78.0*	547.0*	35.1	40.0
10.0 (0.1650)	52.0*	340.3*	48.4	54.6

* Results which were significantly different (based on Welch-t test for inhomogeneous variances with Bonferroni-Holm adjustment) from the control

The ErC_{50} and ErC_{10} values are presented in Table 50 and the EyC_{50} and EyC_{10} values in Table 51. The most sensitive endpoint was total frond area of plants resulting in a 7 d ErC_{50} value of 0.113 mg a.s./L, based on geomean measured concentration of desmedipham and the lowest ErC_{10} was 0.0114 mg a.s./L.

Table 50 Effects on mean Growth Rate of frond number and frond area of *Lemna gibba*

Endpoint (0-7 day)	Frond number (mean growth rate)		Frond area (mean growth rate)	
	nominal conc.	geomean measured conc.	nominal conc.	geomean measured conc.
EC_{10} [mg/L] (95% confidence interval)	0.2881 (0.2042 - 0.3802)	0.0114 (0.066 - 0.0162)	0.3556 (0.2794 - 0.4383)	0.0127 (0.083 - 0.0171)
EC_{20} [mg/L] (95% confidence interval)	1.0394 (0.8503 – 1.2353)	0.0274 (0.020 - 0.0347)	1.0553 (0.9071 – 1.2057)	0.0269 (0.0207 - 0.0330)
EC_{50} [mg/L] (95% confidence interval)	12.101 (9.7598 – 15.702)	0.1463 (0.1074 - 0.2333)	8.411 (7.3348 – 9.8317)	0.1131 (0.0897 - 0.1553)
LOEC [mg/L]	0.625	0.0208	0.313	0.0149
NOEC [mg/L]	0.313	0.0149	0.156	0.0107

Table 51 Effects on Yield of frond number and frond area of *Lemna gibba*

Endpoint (0-7 day)	Frond number (yield)		Frond area (yield)	
	nominal conc.	geomean measured conc.	nominal conc.	geomean measured conc.
EC_{10} [mg/L] (95% confidence interval)	0.0617 (0.0395 - 0.0881)	0.0056 (0.0031 - 0.0081)	0.0442 (0.0301 - 0.0606)	0.0050 (0.0032 - 0.0068)
EC_{20} [mg/L] (95% confidence interval)	0.1795 (0.1312 – 0.2318)	0.0103 (0.0069 - 0.0134)	0.1266 (0.0963 – 0.1593)	0.0089 (0.0065 - 0.0110)
EC_{50} [mg/L] (95% confidence interval)	1.3833 (1.1754 – 1.6369)	0.0328 (0.0273 - 0.0405)	0.9477 (0.8244 – 1.0902)	0.0264 (0.0230 - 0.0306)
LOEC [mg/L]	1.250	0.0295	≤ 0.078	≤ 0.0074
NOEC [mg/L]	0.313	0.0208	< 0.078	< 0.0074

The study fulfilled the validity criteria for control growth, the pH-value did not increase by more than 1.5 units and temperature was within 24 ± 2 °C set in OECD TG 221 (2006). There was a minor accident in the documentation of the study. The documentation of 7-10 days old pre-culture is missing from the study. Photodocumentation was used to check that healthy and suitable plants were used for the performance of the test. Since the validity criteria for control growth was met, this deviation is not considered to have any impact on the test. The study is considered valid and a 7 d ErC_{50} value of 8.41 mg a.s./L was obtained, based on nominal concentrations of desmedipham, and a 7 d ErC_{50} value of 0.113 mg a.s./L based on geometric mean measured concentrations of desmedipham. The NOErC value was 0.0107 mg a.s./L based on geometric mean measured concentrations of desmedipham.

Study 5 – Desmedipham (Myriophyllum spicatum)

2013, 2016, Toxicity of desmedipham technical to the aquatic macrophyte, *Myriophyllum spicatum*, M-461454-01, M-545827-01-1, RAR B.9.2.7/08

The effect of desmedipham technical (purity 99.5 % w/w) on aquatic macrophyte, *Myriophyllum spicatum* was studied in a semi-static toxicity test (higher tier study based on OECD 221). Following a seven-day acclimation period, *Myriophyllum spicatum* shoots were exposed via the water phase to the test item for 14

days. There were four solution renewals during the exposure period, which occurred on exposure days 4, 6, 8 and 11, respectively. Nominal (mean measured) concentrations were control, solvent control (0.1 mL acetone/L), 15 (15.2), 48 (44.0), 150 (125), 490 (435), 1560 (1474) and 5000 (4175) µg a.s./L, respectively. The test system consisted of three replicates per treatment group with three plants each. Shoots within a replicate were planted in the artificial sediment spiked with nutrients (20 XAAP media). Measured parameters were growth rate and yield (NOEC, LOEC and EC50) of total shoot lengths, total plant wet weight and total plant dry weight.

The environmental conditions were 16 hours of light and 8 hours dark, with a light intensity of 7980 to 10720 Lux (mean 9160 Lux), a temperature of 24 ± 0.5 °C, a pH of 8.1 to 9.8 and dissolved oxygen of 7.8 to 11.5 mg/L. Quantitative amounts of desmedipham and its metabolite EHPC were measured in all freshly prepared test levels on day 0, 4, and 11 and additionally in all aged test levels on day 4, 11, and 14 of the exposure period.

Mean measured recoveries for sum of desmedipham and EHPC measured from representative samples of new and old solutions ranged from 83 to 101% of nominal concentrations. Results are based on both nominal test concentrations of desmedipham and geometric mean measured concentrations of desmedipham. The measured recoveries showed that desmedipham was completely hydrolysed in the old solutions. Sediment samples were not collected for chemical analysis.

Table 52 Geometric mean measured concentrations of desmedipham

Sampling time (d)	**Measured concentrations (3 sampling intervals)**					
nom.conc. (mg/l)	**0.015**	**0.048**	**0.15**	**0.49**	**1.56**	**5**
0	0.01514	0.04645	0.12382	0.45404	1.4149	3.31263
4	0.00025	0.00025	0.00025	0.00025	0.00025	0.00025
4	0.01564	0.04522	0.13183	0.45969	1.4371	3.70608
11	0.00025	0.00025	0.00025	0.00025	0.00025	0.00196
11	0.01574	0.04631	0.13967	0.45375	1.4851	3.54911
14	0.00025	0.00025	0.00025	0.00025	0.00025	0.00025
geomean conc.	**0.0020**	**0.0034**	**0.0057**	**0.0107**	**0.0190**	**0.0499**

Highlighted cell measured value below LOQ (0.0005 mg/l) so half the LOQ is used for calclulations

Plants in the control, solvent control and all treatment groups appeared normal throughout the study. Active growth of the control plants during the 14-day exposure period was demonstrated by an average total shoot length yield of approximately 28.3 cm. Replicate A in the solvent control group was identified as an outlier during the statistical analysis and was removed from the data. Growth data for all other plants was included in the data analysis.

Results for growth rates for total shoot length, total plant wet weight and total plant dry weight analyzed at test termination on study day 14 are given in the following table.

Table 53 Inhibition of growth rates of *Myriophyllum spicatum*

Nominal concentration [µg/L]	**Length Growth Rate (cm^{-1})**	**Inhibition of growth rate for total shoot length (%)**	**Wet Weight Growth Rate (g^{-1})**	**Inhibition of growth rate for total plant wet weight (%)**	**Dry Weight Growth Rate (g^{-1})**	**Inhibition of growth rate for total plant dry weight (%)**
Pooled control	0.1139	-	0.1192	-	0.1318	-
15	0.1012	11.1	0.1045	12.3	0.1193	9.5
48	0.0942	17.3*	0.1026	13.9	0.1073	18.6
150	0.0928	18.5*	0.1039	12.8	0.1226	7.0
490	0.0950	16.6*	0.1155	3.1	0.1239	6.0

Nominal concentration [µg/L]	Length Growth Rate (cm^{-1})	Inhibition of growth rate for total shoot length (%)	Wet Weight Growth Rate (g^{-1})	Inhibition of growth rate for total plant wet weight (%)	Dry Weight Growth Rate (g^{-1})	Inhibition of growth rate for total plant dry weight (%)
1560	0.0832	26.9*	0.0880	26.2*	0.0933	29.2*
5000	0.0807	29.2*	0.0729	38.9*	0.0822	37.7*

* Statistically significant difference from pooled controls (Dunnett's one-tailed test; $p < 0.05$).

The ErC_{50} values for each of the three endpoints were determined to be greater than the highest test concentration. The lowest NOErC in the 14-day exposure of *Myriophyllum spicatum* was obtained for shoot length growth rate. The statistical NOErC, LOErC and ErC_{50} for this endpoint were 15, 48 and >5000 µg/L, respectively, based on nominal concentrations of desmedipham.

Table 54 Toxicity of desmedipham technical to *Myriophyllum spicatum*

Test substance		**Desmedipham technical**		
Test object		***Myriophyllum spicatum***		
Exposure		**14 day – Static renewal exposure**		
Endpoint Unit		**[µg/L]**		
Endpoint results		**Day 14 Shoot Length**	**Day 14 Wet Weight**	**Day 14 Dry Weight**
NOEC	Growth rate	15	490	490
	Yield	< 15	490	490
LOEC	Growth rate	48	1560	1560
	Yield	15	1560	1560
EC_{50} (95% C.I.)	Growth rate	> 5000	> 5000	> 5000
	Yield	4046 (not calculable)	2939 (not calculable to 6616)	1518 (688 to not calculable)

The study was performed according to the OECD TG 221 (Lemna growth inhibition test), but the study followed in principle the OECD TG 239 (2014; Water-sediment *Myriophyllum spicatum* toxicity test) except that the replication was smaller than recommended by OECD TG 239 (2014): six for control and four for test levels. However, the study was performed before the test guideline adoption in 2014 and the replication in the test allowed statistical significance to be observed even at second lowest treatment level. The pH range increased more than the required ± 1.5. This is however not considered to invalidate the study since the criteria for control growth was met. Otherwise the study fulfilled the criteria set in OECD TG 239 (2014) and therefore the study was considered valid. The 14 day ErC_{50} value was >5 mg a.s./L, based on nominal concentrations of desmedipham and >0.05 mg a.s./L based on geometric mean measured concentrations of desmedipham. The 14 day NOErC value was 0.002 mg a.s./L based on geometric mean measured concentrations of desmedipham.

Studies with the metabolite (EHPC, Aniline, Phenol)

All metabolites were at least an order of magnitude less toxic to aquatic macrophytes than the parent substance desmedipham. Therefore, the studies with the metabolites presented in the Table 43 are not described here in detail (for more details see the annexed RAR of desmedipham).

11.5.4 Acute (short-term) toxicity to other aquatic organisms

11.6 Long-term aquatic hazard

Evaluation of aquatic chronic hazard for desmedipham is based on studies which are considered valid in the Renewal Assessment Report of desmedipham (RAR annexed to this CLH proposal). All valid studies are presented in the table below and relevant studies for the classification purpose are also summarised below. More details can be found in the annexed RAR.

Table 55: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
Chronic toxicity to fish - Desmedipham					
EU Directive 91/414 EEC; Regulation (EC) No. 1107/2009 US EPA OCPP 850.1400 ELS flow-through 92 d (60 d post hatch) GLP	*Oncorhynchus mykiss* (rainbow trout)	Desmedipham technical Purity 99.5 % w/w	**EC_{10} 0.146 mg/l** Endpoint Growth, EC10 (based on arithmetic mean measured concentrations) **key study**	The study followed the recommendations of OECD TG 210. Malfunction of the diluter was noticed on day 83 and corrected. The deviation is not considered to have on impant on the study results.	2014, 2016 M-482005-01-1 M-545521-01-1 M-582216-01-1 RAR B.9.2.2.1/01
Chronic toxicity to fish - Metabolite EHPC					
EU Directive 91/414 EEC; Regulation (EC) No. 1107/2009 OECD 210 (1992) US EPA OCPP 850.1400 ELS flow through 94 d (62 d post-hatch) GLP	*Oncorhynchus mykiss* (rainbow trout)	EHPC Purity 99.5 % w/w	EC_{10} 3.71 mg/l Endpoint growth, EC_{10} Mean measured recoveries were within the range of 106 to 128% of the nominal concentrations. The results are based on the mean measured concentrations of EHPC.	Study followed the recommendations of OECD TG 210.	2013 M-482025-01 RAR B.9.2.2.1/02
Chronic toxicity to fish - Metabolite Aniline					
ELS flow-through 11.5 d Guideline not stated	*Mocropterus salmoides* (Largemouth bass))	Aniline Purity 99.5%	NOEC 0.051 mg/l (mm) Endpoint growth, NOEC		1979, 2004 M-49247-01-1 RAR B.9.2.2.1/03
Chronic toxicity to fish - Metabolite Phenol					
ELS static-renewal	*Oncorhynchus mykiss* (rainbow trout)	Phenol	NOEC 0.065 mg/l (mm)		1979, 2006 M-491666-01-1 RAR B.9.2.2.1/04

(daily) 30 d No information on the guideline or GLP					
Chronic toxicity to fish - Metabolite Diphenyl urea					
No study was submitted. The acute toxicity studies with Daphnia and algae show that the substance is less toxic than the active substance.					
Chronic toxicity to Daphnids - Desmedipham					
OECD 211 U.S.EPA OPPTS 850.1300, deviation not specified Flow-through 21 d GLP	*Daphnia magna* (cladoceran)	Desmedipham technical Purity 99.5 % w/w	NOEC 0.049 mg/l (based on mean measured concentrations of sum of desmedipham and EHPC) **NOEC 0.020 mg/L** (based on arithmetic mean measured concentrations of desmedipham) **key study**	Fulfils the validity criteria OECD 211 for control mortality and for the mean number of living offspring per parent animal	2012 M-437659-02-1 RAR B.9.2.5.1/03
Chronic toxicity to Daphnids - Metabolite Aniline					
Guideline not stated Flow-through 21 day No GLP	*Daphnia magna* (cladoceran)	Aniline No information on purity	NOEC 0.016 mg/L (based on mean measured concentrations)	For aniline, the endpoints used for risk assessment correspond to the EU-agreed endpoints according to the EU Review Report: Review report for the active substance desmedipham (SANCO/4061/2001- final, 13 February 2004) which have also been used for the EU evaluation of aniline as an existing substance carried out in the framework of Council Regulation (EEC) 793/93 (CSTEE, 2003 and European RAR, 2004).	2004 European RAR RAR B.9.2.5.1/04
Guideline not stated Static-renewal (3x/week)	*Daphnia magna* (cladoceran)	Aniline No information on purity	NOEC 0.004 mg/L (extpolated from the nominal value)	NOEC value extrapolated from a nominal value of 10 µg/l, based on the recovery	2004 European RAR RAR B.9.2.5.1/04

21 d				rate determined at a much higher concentration	
Guideline not stated Semi-static 21 d	*Daphnia magna* (cladoceran)	Aniline No information on purity	NOEC 0.024 mg/L (based on mean measured concentrations)		2004 European RAR RAR B.9.2.5.1/04
Chronic toxicity to Daphnids - Metabolite Phenol					
Static-renewal (3x / week) 16 d No information on guideline or GLP	*Daphnia magna* (cladoceran)	Phenol No information on purity	EC_{10} 0.46 mg/L	For phenol, the endpoints used for risk assessment are based on the EU evaluation of phenol as an existing substance carried out in the framework of Council Regulation (EEC) 793/93 (CSTEE, 2003and European RAR, 2006).	2006 European RAR M-491666-01-1 RAR B.9.2.5.1/05
Chronic toxicity to green algae – Desmedipham					
OECD TG 201 96 h static GLP	*Selenastrum capricornutum (green algae)*	Desmedipham technical Purity 96.8 % w/w	NOEC < 0.05 mg/L	Inhibition on cell growth was already identified in the lowest test concentrations of desmedipham (initial 0.053 mg/L)	1993, 2004 M-146929-02-1 M-146929-02 RAR B.9.2.6.1/02
Chronic toxicity to green algae – Metabolite EHPC					
OECD 201 GLP Static 72 h	*Pseudokirchneriella subcapitata (green algae)*	EHCP (Ethyl-3-hydroxyphenyl carbamate) Purity 97.7 % w/w	ErC_{10} 37 mg/L (based on nominal as measured concentrations stayed within 92-100% of nominal) Endpoint growth rate: E_rC_{10}	Study fulfilled validity criteria. pH range of the test medium was higher than recommended ±1.5 in OECD TG 201 (2011)	2000, 2016 M-197998-01-1 M-197998-01-1 RAR B.9.2.6.1/06
Chronic toxicity to aquatic macrophytes – *Lemna minor*- Desmedipham					
OECD 221, deviation not specified Static-renewal (3x/week) 14 d GLP	*Lemna minor* (duck weed)	Desmedipham technical Purity 97.4 ± 0.22 %	7 d NOEC 0.0492 mg/l Endpoint frond number and dry weight, 7d-NOErC (based on geometric mean measured concentrations of desmedipham)	Fulfilled the validity criteria OECD TG 221	2004, 2016 M-494089-01-1 M-545827-01-1 RAR B.9.2.7/03
Chronic toxicity to aquatic macrophytes – *Lemna gibba*- Desmedipham					
OECD 221 Static-renewal (3x/week) 7 d GLP	*Lemna gibba* (duck weed)	Desmedipham technical Purity 99.5 % w/w	ErC_{10} 0.013 mg/l (based on geometric mean measured concentrations) Endpoint frond area,	Fulfilled the validity criteria OECD TG 221 for control growth, pH did not increase more than 1.5 units, and	2012, 2016, 2017 M-444430-01-1 M-545827-01-1 M-594283-01-1 RAR B.9.2.7/04

			7d-ErC_{10} ErC_{10} 0.011 mg/l (based on geometric mean measured concentrations) Endpoint frond number, 7d-ErC_{10}	the temperature was within 24± 2°C. Minor deviation in the documentation of the study, no impact on the test result.	
Toxicity to aquatic macrophytes – *Myriophyllum spicatum*- Desmedipham					
OECD 221 Static-renewal (days 4,6,8,11) 14 d GLP	*Myriophyllum spicatum* (Eurasian watermilfoil)	Desmedipham technical Purity 99.5 % w/w	**14 d NOErC 0.002 mg a.s/L** (based on geometric mean measured concentrations) Endpoint total shoot length, 14d-NOErC **Key study**	Study was performed according to OECD 221, but followed in principle OECD 239 except the replication was smaller than recommended. pH ranged more than required ±1.5. Criteria for control growth was met. Criteria set in OECD 239 were otherwise met.	2013 M-461454-01-1 RAR B.9.2.7/08
Chronic toxicity to Chironomus riparius - desmedipham					
BBA: (1995); US EPA OPPTS 850.1735 Static 28 d spiked water GLP	*Chironomus riparius* (chironomid)	Desmedipham technical Purity 98.2 % w/w	NOEC 1.0 mg a.s./L (based on initial measured concentrations of the sum of desmedipham and EHPC) 0.14 mg a.s./L (based on initial geometric mean measured concentration of desmedipham)	Desmedipham degrades rapidly in alkaline test medium as in this study (mean pH 7.8)	2002 M-213724-01-1 RAR B.9.2.5.3/01
OECD Proposal for a New Guideline 219 Static 28 d spiked water GLP	Chironomus riparius (chironomid)	Desmedipham Purity 98%	NOEC 7.0 mg a.s./L (based on initial measured concentrations of the sum of desmedipham and EHPC) 3.34 mg a.s./L (based on initial geometric mean measured concentration of desmedipham)	Desmedipham degrades rapidly in alkaline test medium as in this study (mean pH 7.8)	2005 M-494081-01-1 RAR B.9.2.5.3/02
Chronic toxicity to Chironomus riparius -EHPC					
OECD 219	Chironomus	EHPC	EC10 > 5 (nom)	Desmedipham	Thomas et al.

Static 28 d spiked water GLP	riparius (chironomid)	Purity 99.1 % w/w	NOEC 5 (nom) (based on nominal concentrations as the recovery of EHPC in day 0 samples were between 116-120 % of nominal concentrations.)	degrades rapidly in alkaline test medium as in this study (mean pH 7.8)	(2012) M-438278-01-1 RAR B.9.2.5.3/03

[1] Indicate if the results are based on the measured or on the nominal concentration

11.6.1 Chronic toxicity to fish

One valid chronic test (EC_{10} 0.146 mg a.s./L) with desmedipham on fish *Oncorhunchus mykiss* (rainbow trout) and one with each metabolite EHPC, aniline and phenol were available in the RAR. Test results from two prolonged fish test were also presented in the RAR but not evaluated as early life stage toxicity test is preferred for evaluating chronic hazard. Those results are not presented either in this CLH-proposal and only studies which are relevant for classification are summaries below.

Study 1 - Desmedipham

2014, 2016. Early life stage toxicity of desmedipham technical to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions. M-482005-01, M-545521-01-1, M-582216-01-1, RAR B.9.2.2.1 /01

Chronic toxicity of desmedipham technical (purity 99.5 % w/w) to the rainbow trout was studied in an early life stage test according to guidelines EU Directive 91/414/EEC, Regulation (EC) No. 1107/2009 US EPA OCSPP 850.1400). Rainbow trout (starting with eggs at <24 hours old) were exposed to desmedipham in a flow-through system over a period of 92 days. Test vessels (4 replicates/treatment) were dosed via a modified proportional diluter with a renewal rate of approximately 10 turnovers/day. Nominal concentrations were 0.0667, 0.120, 0.216, 0.389 and 0.700 mg a.s./L and controls dilution water and solvent control: acidified Dimethylformamide (2% phosphoric acid solution, pH < 4) at 0.1 mL/L. The test concentrations based on arithmetic mean measured concentrations of desmedipham were 0.076, 0.115, 0.197, 0.405 and 0.683, respectively. Test conditions were following: temperature 10.1°C to 10.7 °C (mean: 10.3 °C), photoperiod: 16 hours light / 8 hours dark with 30 minutes dawn/dusk, light intensity: 488 to 764 Lux, pH: 7.4 to 8.0, water hardness: 46 mg/L as CaCO3, and dissolved oxygen: 9.0 to 11.2 mg/L (82 to 102% oxygen saturation).

Measured parameters were: sublethal effects, fish hatchability (days to hatch and numbers hatched), swim-up behavior, survival (post-hatch success) and growth (standard length and dry weight for all surviving fish on Day 92). Results are shown in the following table.

Table 56 Toxicity of desmedipham to fish (ELS), based on artihmetic mean measured concentrations of desmedipham

Test Substance	**Desmedipham**	
Test Object	***Oncorhynchus mykiss***	
Exposure	**92 days (60 days post-hatch), flow-through (ELS)**	
Fry Survival (Day 92):	NOEC: 0.115 mg a.s./L	LOEC: 0.197 mg a.s./L
Percent Hatch / Time to Hatch:	NOEC: 0.405 mg a.s./L	LOEC: 0.683 mg a.s./L
Percent Swim-up / Time to Swim-up:	NOEC: 0.197 mg a.s./L	LOEC: > 0.197 mg a.s./L
Growth (Standard Length):	NOEC: 0.076 mg a.s./L	LOEC: 0.115 mg a.s./L

Test Substance	**Desmedipham**	
Test Object	***Oncorhynchus mykiss***	
Exposure	**92 days (60 days post-hatch), flow-through (ELS)**	
Growth (Dry Weight):	NOEC: 0.076 mg a.s./L	LOEC: 0.115 mg a.s./L EC_{10}: 0.146 mg a.s./L
Morphological & Behavioural Effects:	NOEC: 0.115 mg a.s./L	LOEC: 0.1.97 mg a.s./L

The study followed the recommendations of OECD TG 210. The validity with regards to hatching success (≥75%) and post hatch success (≥75%) were fulfilled and the mean hatching success in the controls was 91.0%, corrected for viability. Dissolved oxygen was > 82 % of the air saturation value and the water temperature did not differ by more than 1.5°C between test chambers or between successive days at any time during the test, and was within the temperature ranges specified for the test species.

On day 83 malfunctioning of the diluter were noticed. Due to a malfunction of the diluter system the compound was not injected into the test system for approximately 15 hours. The malfunction was corrected and the diluter was cycled manually 69 times in order to re-establish nominal test concentrations. Water samples taken after the diluter malfunction was corrected and showed recoveries within the expected range. As the malfunction period was a short time compared to the full length of this study (< 1%) and because this incident occurred one week before study termination with no critical development stage involved, this deviation is not considered to have an impact on the study results. The lowest EC_{10} value of 0.146 mg a.s./L is used based on arithmetic mean measured concentration.

Study 2 – EHPC

2014. Early life stage toxicity of EHPC to the rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions, M-482025-01, RAR B.9.2.2.1 /02

Rainbow trout eggs starting at <24 hours old were used during this early life stage test to study chronic toxicity of metabolite EHPC (purity 99.8 & w/w). The study duration was 94 days (62 days post-hatch) under flow through conditions. Test vessels were dosed via a modified proportional diluter with a renewal rate of approximately 10 turnovers/day. Nominal test concentrations were: dilution water control, 0.625, 1.25, 2.50, 5.00 and 10.0 mg p.m. /L (pure metabolite). Mean measured recoveries were within the range of 106 to 128% of the nominal concentrations.

Test conditions were: temperature: 10.8°C to 11.5 °C (mean: °C), photoperiod: 16 hours light / 8 hours dark with 30 minutes dawn/dusk, light intensity: 610 to 771 Lux, pH: 7.7 to 8.1, water hardness: 48-68 mg/L as CaCO3, and dissolved oxygen: 8.9 to 11.2 mg/L (81 to 102% oxygen saturation). Measured parameters were: sublethal effects, fish hatchability (days to hatch and numbers hatched), swim-up behavior, survival (post-hatch success) and growth (standard length and dry weight for all surviving fish on Day 94). Results are shown in the following table. The results are based on the mean measured concentrations of EHPC.

Table 57 Toxicity of EHPC to fish (ELS)

Test Substance	**EHPC**		
Test Object	***Oncorhynchus mykiss***		
Exposure	**94 days (62 days post-hatch), flow-through (ELS)**		
Fry Survival (Day 94):	NOEC: ≥ 10.6 mg p.m./L	LOEC: > 10.6 mg p.m./L	---
Percent Hatch / Time to Hatch:	NOEC: ≥ 10.6 mg p.m./L	LOEC: > 10.6 mg p.m./L	EC_{10} (95% C.I.): 9.15 (NA) mg p.m./L
Percent Swim-up / Time to Swim-up:	NOEC: ≥ 10.6 mg p.m./L	LOEC: > 10.6 mg p.m./L	---
Growth (Standard Length):	NOEC: 2.86 mg p.m./L	LOEC: 5.60 mg p.m./L	EC_{10} (95% C.I.): 13.2 (10.7 – 15.7) mg p.m./L

Growth (Dry Weight):	NOEC: 2.86 mg p.m./L	LOEC: 5.60 mg p.m./L	EC_{10} (95% C.I.): 3.71 (3.02 – 4.39) mg p.m./L
Morphological & Behavioral Effects:	NOEC: 2.86 mg p.m./L	LOEC: 5.60 mg p.m./L	The NOEC and LOEC were empirically determined.

p.m./L = pure metabolite/L; NA = not applicable
--- = Due to the nature of the data, the EC_{10} could not be calculated

The 94-day exposure to EHPC resulted in a NOEC of 2.86 mg p.m./L and a LOEC of 5.60 mg p.m./L based on growth (length and dry weight) and morphological/behavioral effects. The lowest EC_{10} was estimated for the growth parameter (dry weight) at 3.71 mg p.m./L. Endpoints were based on mean measured concentrations of EHPC.

The study followed the recommendations of OECD TG 210. The validity with regards to hatching success (≥75%) and post hatch success (≥75%) were fulfilled and the mean hatching success in the controls was 97.3% corrected for viability. Dissolved oxygen was > 81 % of the air saturation value and the water temperature did not differ by more than 1.5 °C between test chambers or between successive days at any time during the test, and was within the temperature ranges specified for the test species.

Study 3 – Aniline

2004. European RAR. The EU evaluation of aniline as an existing substance carried out in the framework of Council Regulation (EEC) 793/93 (European RAR, 2004) and listed in the EU Review Report for Desmedipham (SANCO/4061/2001 - final, 13 February 2004), M-492497-01-1, RAR B.9.2.2.1/03

Aniline has been evaluated in the Framework of Council Regulation (EEC) 793/93. In the RAR of desmedipham two studies for fish early life stages from the EU Risk Assessment Report on Aniline were presented shortly. An old study from 1979 examined the long-term toxicity of aniline in an embryo-larval test with the largemouth bass *Micropterus salmoides* as test organism with hard, respectively soft water. In a flow-through system (temperature: 19-24°C; dissolved oxygen: 7.7-9 mg/L; water hardness: 50, respectively 200 mg/L CaCO3; pH: 7.3-8.1) eggs were exposed to the test substance 1-2 hours after spawning. Exposure was maintained through 4 resp. 8 days after hatching giving exposure periods of 6.5-7.5 resp. 10.5-11.5 d. Aniline concentration was measured daily. Test parameters were egg hatchability and survival 4 and 8 days post hatching. From the available test a NOEC value of 45 µg/L for hard and 51 µg/L for soft water, respectively, was roughly estimated.

Although it was stated in the RAR that it is impossible to check whether the above described study is performed according to the OECD TG 210, and it was also argued whether this study should be considered more like an extended embryo test due to the short post hatch exposure period of 4-8 days (to fulfill the OECD 210 recommendation) than ELS study, it was nevertheless accepted to use the endpoint of NOEC 51 µg/L. In this CLH-proposal this study is, however, used only as for information due to above mentioned discrepancies.

Study 4 – Phenol

E**uropean Chemicals Bureau, Institute for Health and Consumer Protection, European Union Risk Assessment Report on Phenol (CAS: 108-95-2) Volume 64, M-491668-01, RAR B.9.2.2.1 /04**

As with aniline in the RAR of desmediphan, an old study from the year 1979 has been chosen the most relevant among studies presented in the EU Risk Assessment Report on Phenol. The long-term toxicity of phenol in an embryo-larval test with *Oncorhynchus mykiss* was studied in hard and soft water in a flow-through system (temperature: 12-14 °C; dissolved oxygen: 9-11 mg/L; water hardness: 50 and 200 mg/L CaCO3; pH: 7.3-8.1). Eggs were exposed to the test substance 20 minutes after fertilisation. Exposure was maintained through 8 days after hatching. Average hatching time for *Oncorhynchus mykiss* was 22 days. Phenol concentration was measured daily (no results presented). Log probit analysis was used by the authors to determine the LC_{10} and LC_{50} at hatching and 8 days after hatching. EC_{10}-values (for survival) of 2 µg/L for

hard water and of 65 µg/L for soft water could be determined that were regarded as NOEC values for 22-30-days exposure. The same comments applied to this study as in the case of aniline above. More details in annexed RAR.

11.6.2 Chronic toxicity to aquatic invertebrates

One valid long term toxicity test is available for *Daphnia magna* with the NOEC value of 0.020 mg a.s./L. Two other studies were considered not reliable either due to lack of measured concentrations or the validity criteria set in OECD TG 211 (2013) was not met. For metabolite aniline even lower NOEC values 0.004 – 0.024 mg/L were presented in the RAR. These values had been extracted from the EU RAR on Aniline and the validity of any of these studies could not be checked.

Study 1 - Desmedipham

2012, 2017. Desmedipham: A flow-through life-cycle toxicity test with the cladoceran (*Daphnia magna*) - Final report M-437659-02. M-582262-01-1, RAR B.9.2.5.1/03

Chronic toxicity of Desmedipham technical (99.5% w/w) was studied during 21 days in flow-through test according to guidelines OECD 211; USEPA (=EPA): OPPTS 850.1300. Daphnids (neonates, less than 24 hours old) were exposed to nominal test concentrations of 6.3, 13, 25, 50 and 100 µg a.s./L and to a negative control (dilution water) and a solvent control (0.05 mL/L dimethylformamide) in a flow-through system. The arithmetic mean measured concentrations of desmedipham were 4.1, 6.3, 10, 20 and 38 µg a.s./L. Two replicate test chambers were tested for each treatment and control group. Each replicate contained two compartments with five daphnids each, resulting in a total of 20 daphnids in each treatment and control group. Observations of the effects of desmedipham on survival, reproduction and growth were used to determine NOEC, LOEC and the maximum acceptable toxicant concentration (MATC).

Light intensity at test initiation was 318 Lux at the surface of the water and temperature was 20 ± 1°C, pH 8.0-8.1 and oxygen 6.8-8.4 mg O2/L. Water samples were collected from alternating replicate test chambers in each treatment and control group at the beginning of the test, at appr. weekly intervals during the test and at the end of the test to measure concentrations of test substance. All samples were collected from mid-depth, placed in glass vials, acidified with two drops of 10 % phosphoric acid and processed immediately for analysis. Samples were analysed using HPLC with UV detection at 240 nm. The LOD for desmedipham was 0.0465 µg a.i./L and for the EHPC metabolite 0.0882 µg a.i./L.

Table 58 Summary of survival, reproduction and growth of *Daphnia magna* exposed to desmedipham for 21 days

Arithmetic mean measured concentration of DMP (µg a.s./L) (nom)	Percent adult survival [1]	Mean no. neonates per reproductive day ± std. dev.	Mean length ± std. dev. (mm)	Mean dry weight ± std. dev. (mg) [1]
Control	95.0	11.3 ± 1.40	5.0 ± 0.10	1.08 ± 0.105
Solvent Control	100	10.3 ± 0.84	4.8 ± 0.19	0.93± 0.071
Pooled Control	97.5	10.8 ± 1.19	4.9 ± 0.18	1.01 ± 0.116
4.1 (6.3)	100	9.4 ± 0.75	4.9 ± 0.12	0.96 ± 0.062
6.3 (13)	95.0	11.2 ± 1.33	5.0 ± 0.09	0.96 ± 0.033
10 (25)	100	9.1 ± 0.67	4.9 ± 0.18	1.03 ± 0.108
20 (50)	95.0	10.0 ± 1.69	5.0 ± 0.14	0.91 ± 0.130
38 (100)	60.0*	2.2 ± 1.45*	4.6 ± 0.21*	0.94 ± 0.095

* Indicates a statistically significant decrease in comparison to the pooled control ($p \leq 0.05$).
[1] There were no statistically significant decreases in survival in comparison to the pooled control using Dunnett's test ($p > 0.05$).

The mean dry weight is higher than in the solvent control and it is reduced by around 6% in comparison with pooled controls. Therefore, the EC_{10} and EC_{20} for dry weight are higher than the 38 µg/L. No statistical calculations were performed for dry weight. Regarding number of offspring, immobility of adults, and length at the end of the study, effects were observed at the highest concentration only. Consequently, no significant dose-response relationship is observed and no valid EC_{10} or EC_{20} values can be calculated for these endpoints.

The NOEC for growth (based on length), survival and reproduction was 20 µg a.s./L, the LOEC was 38 µg a.s./L based on arithmetic mean measured test concentrations of desmedipham.

The study was considered to fulfil the validity criteria set in OECD TG 211 (2012) for control mortality and for the mean number of living offspring per parent animal. A NOEC value of 0.049 mg/L was obtained based on mean measured concentrations of the sum of desmedipham and EHPC and 0.020 mg a.s./L based on arithmetic mean measured concentration of desmedipham.

Study 2 – Aniline

EU evaluation of aniline as an existing substance carried out in the framework of Council Regulation (EEC) 793/93 (CSTEE, 2003) has presented results from three chronic 21 d study with *Daphnia magna*. Those test results were also presented in the RAR of desmedipham without any detailed descriptions: 1) a flow-through study (1989) with *Daphnia magna* with a 21 day NOEC value of 16 µg/L (based on mean measured concentrations); 2) a semi-static study (1988) (three renewals per week) with *Daphnia magna* with a 21 day NOEC value of 4 µg/L (extrapolated from nominal concentrations); 3) a semi-static study (1988) with *Daphnia magna* with a 21 day NOEC value of 24 µg/L based on mean measured concentrations. According to the evaluation in the EU Risk Assessment Report "None of these tests was conducted according to international guidelines but careful examination of the test reports allows the conclusion that they can all be regarded as valid (with restriction) and that it cannot be justified to prefer one of the tests. It could be stated that the NOEC of 4 µg/l derived from the study from the year 1988 is not as reliable as the other 2 daphnia tests, because this value was extrapolated from a nominal value of 10 µg/l, based on the recovery rate that was determined at a much higher concentration."

In the RAR it is mentioned that the validity of any of these studies could not be checked.

Study 3 – Phenol

European Chemicals Bureau, Institute for Health and Consumer Protection, European Union Risk Assessment Report on Phenol (CAS: 108-95-2), Volume 64, M-491668-01, in RAR Vol 3, B.9.2.5.1 /05

As with aniline the results presented in EU Risk assessment report are provided here: 1) a study with *Ceriodaphnia dubia* with 4 day and 7 d values of 1.77 mg/L and > 5 mg/L (geometric means of NOEC and LOEC), respectively, for reproduction 2) a study with *Ceriodaphnia dubia* with 4 day and 7 d values of 4.9 mg/L for reproduction 3) a study with *Ceriodaphnia dubia* with a 8 day NOEC of 0.84 mg/L for survival and 6.5 mg/L for reproduction and with *Daphnia magna* with a 11 day NOEC of 0.5 mg/L for survival and 0.8 mg/L for reproduction 4) a study with *Daphnia magna* with 16 d EC_{10}-value of 0.46 mg/L and an EC_{50}-value of 10 mg/L was found for the endpoint growth. The endpoints are based on nominal concentrations.

The lowest endpoint of EC_{10} 0.46 mg/l was used in the RAR of desmedipham since all four studies showed the same magnitude of effect. In this study daphnids (< 24-hour-old) were exposed to different phenol concentrations for 16 days and they were fed green algae each day. Three times a week daphnids were transferred in new test solution and all newly born daphnids were removed. After 16-days the length from the top of the head to the end of the tail was measured using binoculars equipped with an ocular micrometer. Validity criteria were not discussed in the report and the test concentrations were not measured.

Only a short summary had been presented in the EU report for phenol. None of these studies can be considered to fulfil the validilty criteria since there is no information on the analytical results and the exposure period is shorter than required in OECD TG 211 (21 days).

11.6.3 Chronic toxicity to algae or other aquatic plants

One green algae test was available for desmedipham with the NOEC value < 0.05 mg/L. The most sensitive species was aquatic macrophyte *Myriophyllum spicatum* with the 14d-NOErC value of 0.002 mg/L. Also studies with *Lemna gibba* and *Lemna minor* demonstrated desmedipham to be hazardous to aquatic macrophytes on the long-term as the ErC_{10}/NOEC values were < 0.1 mg/L.

Study 1 – Desmedipham

1993, 2004. Toxicity of Desmedipham technical to the green algae, Selenastrum capricornutum. M-146929-01-1, M146929-02, RAR B.9.2.6.1/02

This study is already summarised in chapter 11.5.3. The NOEC was less than 0.05 mg/L as the inhibition on cell growth was already identified in the lowest test concentrations of desmedipham (at the initial 0.053 mg/l).

Study 2 – Desmedipham (Lemna minor)

2004, 2016. Desmedipham technical – Aquatic plant toxicity test Lemna minor, semi-static, 14d. M-49089-01-1, M-545827-01-1, RAR B.9.2.7/03

This study is already summarised in chapter 11.5.3. The 7-d NOErC value of 0.0492 mg a.s./L (frond number and dry weight) for aquatic macrophyte *Lemna minor* was determined based on geometric mean measured concentrations of desmedipham.

The study fulfilled the validity criteria for control growth and the pH-value was within the acceptable limits set in OECD TG 221 (2006). The original water temperature measurements were not presented, but the temperature was stated to be within 24 ± 2 °C. This missing information is not considered to invalidate the study since control growth fulfilled the validity criteria and hence the study is considered valid.

Study 3 – Desmedipham (Lemna gibba)

2012, 2016, 2017. Lemna gibba G3 growth inhibition test with desmedipham (technical) under semi-static conditions. M-444430-01, M-545827-01-1, M-594283-01-1, RAR B.9.2.7/04

This study is already summarised in chapter 11.5.3. The 7-d ErC_{10} value of 0.011 mg a.s./L (frond number) and 7-d ErC_{10} value of 0.013 mg a.s./L based on geometric mean measured concentrations of desmedipham were obtained.

The study fulfilled the validity criteria for control growth, the pH-value did not increase by more than 1.5 units and temperature was within 24 ± 2 °C set in OECD TG 221 (2006). There was a minor accident in the documentation of the study. The documentation of 7-10 days old pre-culture is missing from the study. Photodocumentation was used to check that healthy and suitable plants were used for the performance of the test. Since the validity criteria for control growth was met, this deviation is not considered to have any impact on the test.

Study 4 – Desmedipham (Myriophyllum spicatum)

2013, 2016, Toxicity of desmedipham technical to the aquatic macrophyte, Myriophyllum spicatum, M-461454-01, M-545827-01-1, RAR B.9.2.7/08

This study is already summarised in chapter 11.5.3. The 14 day NOErC value was 0.002 mg a.s./L based on geometric mean measured concentrations of desmedipham.The study was performed according to the OECD TG 221 (Lemna growth inhibition test), but the study followed in principle the OECD TG 239 (2014; Water-sediment *Myriophyllum spicatum* toxicity test) except that the replication was smaller than recommended by OECD TG 239 (2014): six for control and four for test levels. However, the study was performed before the test guideline adoption in 2014 and the replication in the test allowed statistical significance to be observed even at second lowest treatment level. The pH range increased more than the required ± 1.5. This is, however, not considered to invalidate the study since the criteria for control growth was met. Otherwise the study fulfilled the criteria set in OECD TG 239 (2014). **This study is considered as a key study to derive aquatic chronic classification for desmedipham.**

11.6.4 Chronic toxicity to other aquatic organisms

Study 1 – Desmedipham

2002. 2016. Chronic toxicity to the sediment dwelling chironomid larvae *Chironomus riparius* Desmedipham Code: AE B038107 00 1D98 0007, M-213724-01-01, M-545523-01-1, RAR B.9.2.5.3/01

Chronic toxicity of desmedipham technical (purity 98.2 % w/w) to *Chironomus riparius* was studied during 28 days under a static test condition. Sediment dwelling larvae were exposed to nominal test concentrations of 0, 0 (solvent control; acetone 0.1 ml/L), 0.125, 0.25, 0.5, 1.0 and 2.0 mg/L in overlying water. Four replicates were used in each treatment level and in control groups. Into each vessel, 25 2-3 day old larvae were placed one day prior to application of the test item. The sex and number of emerged midges were recorded. The test vessels were observed three times per week for any visual differences compared with the control. During the period of expected emergence test vessels were checked daily for emerged midges. The sediment used in the test was composed of 10 % sphagnum peat moss, 20 % kaolin clay, and 70 % industrial quartz sand. Calcium carbonate (CaCO3) were used to adjust the pH of this medium to 7.2.

Samples taken from the water column of the test vessels on days 0, 7 and 21 were analysed for the concentrations of DMP and its hydrolysis metabolite EHPC by HPLC/UV. Samples were taken from the top, the middle and the lowest concentration on day 0 and from all concentrations on days 7 and 21. LOQ in water phase was 17.53µg/L. Test water was an artificial modified mineral medium M4 with temperature range of of 19.7 to 20.2 °C, mean oxygen content of 8.1 mg/L and mean pH of 7.8. Optimum light cycle was used.

The study was consided to fulfill the OECD TG 219 validity criteria for emergence in control as well as the criteria for pH, oxygen concentration and temperature range in the RAR of desmedipham. The pH of the artificial sediment was higher (7.2) than recommended by the guideline (6.5 ± 0.5). This was not considered to invalidate the study. OECD test guideline recommends a static test design for Chironomous study. Desmedipham degraded quickly in this study to EHPC and both compounds were not detected in the higher concentrations in the end of the study period showing fast degradation of both test compounds at the test pH. Based on initial desmedipham concentration a NOEC value of 1.0 mg/L was obtained. RMS requsted that the results should also be based on geomean measured intial concentration. Since the NOEC-level was not analysed at test initiation, the mean recovery of all concentration levels was used to re-calculate the NOEC-level, using the geomean of initially measured concentrations, i.e. 14% * 1.0 mg a.s./L = 0.14 mg a.s./L

Study 2 – Desmedipham

2005, 2016. Desmedipham: A prolonged sediment toxicity test with *Chironomus riparius* using spiked water - Final report. M-494081-01. M-545523-01-1, RAR B.9.2.5.3/02

In this prolonged sediment toxicity study groups of 20 midges in four replicates (total 80 midges in each treatment and control group) were exposed to five test concentrations of desmedipham technical (purity 98 %), 0.44, 0.88, 1.8, 3.5 and 7.0 mg a.s./L, a negative control (dilution water) and a solvent control (0.1 ml acetone/L) for 28 days. The test chambers were observed three times per week during the first 13 days of the test to make visual assessments of any abnormal behaviour (e.g., leaving sediment, unusual swimming). During the period of expected emergence following Day 13, the test chambers were observed on a daily basis and the sex and numbers of fully emerged midges were recorded. After identification, the midges were removed from the test chambers.

Also, five additional test chambers were added to each of the negative and solvent controls and the low, middle and high treatment levels for analytical sampling of water and sediment. Samples were analysed LC/MS/MS and HPLC/UV. The sediment used in the test was composed of12.5% sphagnum peat moss, 18% kaolin clay, and 69% industrial quartz sand (to reach the recommended org C). The temperatures were within the 20 ± 2°C, dissolved oxygen concentrations were ≥ 6.6 mg O2/L (73 % of saturation) throughout the test. Measurements of pH ranged from 8.1 to 8.7 in the overlying water and 7.1 to 7.9 in the sediment during the

test. The photoperiod was 16 hours of light and 8 hours dark with a light intensity of 285 Lux at test initiation.

The study fulfilled the OECD TG 219 validity criteria for emergence in solvent control as well as the criteria for pH, oxygen concentration and temperature range and was therefore considered valid. OECD test guideline recommends a static test design for the Chironomous study. Desmedipham was degraded almost completely within 24 h, but the approximate mass balance showed that dosing was correctly performed. Based on nominal concentrations of sum of desmedipham and EHPC a NOEC value of 7.0 mg/L was obtained. Based on geomean of initially measured concentrations the NOEC corresponds to an initially measured concentration of 3.34 mg a.s./L.

Study 3 – Desmedipham

2012. EHPC (metabolite of desmedipham): A prolonged sediment toxicity test with Chironomus riparius using spiked water - Final report, M-438278-01, RAR B.9.2.5.3/03

In this prolonged sediment toxicity study groups of 20 midges in four replicates (total 80 midges in each treatment and control group) were exposed to five nominal test concentrations of EHPC (purity 99.1 % w/w) (0.313, 0.625, 1.25, 2.50 and 5.00 mg p.m../L1), a negative control and a solvent control (acetone) for 28 days under static test conditions. Mortality and emergence as well as signs of abnormal behavior were recorded on a daily basis. Each test chamber contained a quantity of sediment [composed of approximately 5% sphagnum peat moss, 20% silt and clay (kaolin clay) and 75% industrial quartz sand] and overlying water. Four additional replicates were added in each treatment and control group for analytical sampling of sediment and water. The results of the study are based on the nominal and time-weighted test concentrations in the overlying water. The collection and analyses were done on days 0, 14 and 28.

The lighting intensity was 506 Lux at a photoperiod of 16 hours of light and 8 hours of darkness. The water temperature ranged from 19.9 to 20.6°C. The dissolved oxygen was ≥6.8 mg./L (corresponding to 76% of saturation) The water pH ranged from 7.9 to 8.5.

The study fulfilled the OECD TG 219 validity criteria for emergence in the solvent control as well as the criteria for, oxygen concentration and temperature and pH range and was therefore considered valid. The NOEC value is 5 mg p.m./L based on nominal concentrations which was considered acceptable since the recovery of EHPC in day 0 samples were between 116-120 % of nominal concentrations.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Full acute data set was available for desmedipham as there were acute toxicity studies on fish, aquatic invertebrates, algae and aquatic macrophytes. Also studies with metabolites EHPC, aniline and phenol were available for all trophic levels. Metabolite aniline (CAS 62-53-3) has a harmonized classification of Aquatic Acute 1 under CLP. Classification proposal is based on studies conducted with desmedipham as the lowest and the most reliable endpoint values for classification purpose were obtained from studies with the parent substance.

Several studies were disregarded due to instability of desmedipham in the test system. The lowest EC_{50} value for fish was 1.41 mg/L (*Oncorhynchus mykiss*), for aquatic invertebrates 0.35 mg/L (*Daphnia magna*) and for aquatic plant 0.113 mg/L (*Lemna gibba).* 72 h E_rC_{50} value of 0.064 mg/L was estimated for green algae *Selenastrum capricornutum* which was the most sensitive species. Based on the available data it is concluded that desmedipham does fulfil the criteria for classification as **Aquatic Acute Category 1. An M factor of 10** is warranted based on the ***Selenastrum capricornutum* 72 h E_rC_{50} 0.064 mg/L** (M factor 10 when $0.01 < L(E)C_{50} \leq 0.1$).

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Bioaccumulation

The log K_{OW} value for desmedipham (3.5) and for the degradation products aniline (0.9), EHPC (0.87), phenyl (1.47) and diphenyl urea (2.3), measured according to OECD test guidelines 107 and 117, are lower than the CLP log K_{OW} trigger value of ≥ 4 intended to identify substances with a potential to bioaccumulate under CLP criteria. In the experimental studies according to the OECD test guideline 305 "Bioconcentration: Flow-through Fish Test" (1996), the highest BCF_{SS} values in whole fish for desmedipham only was 65 L/kg and for desmedipham and the degradates was 333.9 L/kg (as determined from ^{14}C-labelled constituents). According to the CLP guidance, the BCF value should be based on the radiolabelled parent compound but the BCF values for total radioactivity may be used as well. The values obtained are lower than the CLP trigger value of 500 and, therefore, **desmedipham is considered to have low potential to bioaccumulate.**

Degradation

A **ready biodegradability test** (OECD test guideline 301D "Closed-Bottle-Test") shows only 21 % of desmedipham degrading after 28 days. Therefore, the test suggests desmedipham being not readily biodegradable for purposes of classification as the pass level criteria of ready biogradation test (70 % of DOC removal or 60 % of theoretical oxygen demand) within 28 days was not reached.

Based on the **simulation test in surface water** (OECD test guideline 309 "Simulation biodegradation test"), the calculated half-lives for desmedipham ranged from 6 minutes to 3 hours in natural surface water systems. For degradate diphenyl urea, DT_{50} value of 2.7 days was obtained. However, DT_{50} values for degradate aniline were from 34.9 to 75.9 days. Therefore, the parent compound is not degraded with a half-life of < 16 days and, therefore, the CLP criteria for rapid degradation is not fulfilled.

According to **hydrolysis tests** (OECD test guideline 111 "Hydrolysis as a function of pH"), desmedipham is hydrolytically stable under acidic conditions (DT_{50} from 4884 to 351 days at pH 4) but the hydrolytic degradation will be rapid (DT_{50} from 14 minutes to 26 hours at 20 °C) in neutral and alkaline (pH 6-9) environments such as many natural waters. However, the test results also suggest that the desmedipham degradation products, aniline and EHPC, do not hydrolyse as their concentration increased towards the end of the studies. According to the criteria in CLP guidance, the substance might be considered as rapidly degradable for classification purposes only when the longest half-life determined within the pH range of 4-9 is shorter than 16 days and the hydrolysis products formed do not fulfil the classification criteria as hazardous for aquatic environment. As desmedimedipham is hydrolytically stable under acidic conditions and aniline (CAS 62-53-3) has a harmonized classification as hazardous to the aquatic environment under CLP (Aquatic Acute 1), desmedipham does not fulfil the CLP criteria of being rapidly degradable**.**

The studies on degradation of desmedipham in **water/sediment systems** support the beforementioned observations for desmedipham and its degradation products. Based on the degradation results of **soil degradation** studies, desmedipham might not be rapidly degradable (DT_{50} from 3.7 to 127.2 days) in soil under aerobic conditions. The conclusion is also supported by the **inherent biodegradation test** (OECD test guideline 302C "Modified Miti-test (II)") in which 45 % degradation of desmedipham was achieved after 28 days. Furthermore, **photodegradation** of desmedipham was measured being insignificant in water and soil. For the degradate EHPC, a photodegradation DT_{50} value of 9.2 days was determined in water.

Overall, degradation information does not provide sufficient data to show that desmedipham is ultimately degraded to > 70% within 28 days (equivalent to a half-life of less than 16 days) or being transformed to non-classifiable products. Therefore, desmedipham is considered being **not rapidly degradable** according to the CLP criteria.

Toxicity

Desmedipham is considered being non-rapidly degradable according to the CLP criteria. The adequate chronic toxicity data for desmedipham was available for three trophic levels fish, aquatic invertebrates

including sediment dwelling organisms and aquatic plants. Classification proposal is based on studies conducted with desmedipham although there were acute and chronic studies available for metabolites EHPC, aniline, phenol. The lowest and the most reliable endpoint values for classification purpose were obtained from studies with the parent substance desmedipham.

Several algae studies were disregarded due to instability of the test substance desmedipham in the test system. No exact NOEC/EC_{10} value was available for green algae as in the available study the inhibition on cell growth was already indentified in the lowest test concentrations of desmedipham (at the initial 0.053 mg/l). The desmedipham concentrations dropped dramatically towards the end of the study and were detected only in the two highest test concentrations on day 3. Therefore NOEC was < 0.05 mg/L for algae. The lowest endpoint values were for fish EC_{10} of 0.146 mg/L (*Oncorhynchus mykiss*), aquatic invertebrate NOEC of 0.020 mg/L (*Daphnia magna*), and for aquatic macrophyte 14d-NOErC of 0.002 mg/L (*Myriophyllum spicatum*) which was the most sensitive species. Based on the available data it is concluded that desmedipham does fulfil the criteria for classification as **Aquatic Chronic Cat. 1. An M factor of 10** is warranted based on the ***Myriophyllum spicatum* 14d-NOErC of 0.002 mg/L** (M factor 10 for non-rapidly degradable substance when $0.001 < NOEC \leq 0.01$).

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Conclusions on classification and labelling for environmental hazards of desmedipham.

Hazard Class and Category code(s)	M factor	Hazard Statement
Aquatic Acute Category 1, H400	10	Very toxic to aquatic life
Aquatic Chronic Category 1, H410	10	Very toxic to aquatic life with long lasting effects

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Desmedipham has a current CLP Annex VI classification of Aquatic Acute 1; H400, M = 10 and Aquatic Chronic 1; H410 in Annex VI of CLP. The DS proposes to classify the substance as Aquatic Acute 1; H400, M = 10; Aquatic Chronic 1; H410, M = 10.

Degradation

Hydrolysis

Four studies on hydrolytic degradation for Desmedipham and two for degradant EHPC were considered valid in the RAR. The studies followed the OECD TG 111. The estimated half-lives ranged from 4 884 days at pH 4 to 4 minutes at pH 9 at 20 °C, indicating hydrolysis being strongly dependent on the pH. Based on the results, the hydrolysis will be rapid in neutral and alkaline environments, such as many natural waters. The results also show that the amounts of aniline and EHPC increased towards the end of the studies indicating that these two Desmedipham degradants do not hydrolyse. Degradant EHPC was confirmed being hydrolytically stable since less than 10 % hydrolysis was detected after five days at pH 4-9 at

50 °C.

Photolysis

Six studies on photochemical degradation in water for Desmedipham, conducted according to the OECD TG 318, were considered valid in the RAR. The direct photolysis of Desmedipham was shown to be insignificant, as no photodegradation occurred after several days of continuous exposure at pH 4-5.

Soil and air photolysis studies are also available in the RAR. Any Desmedipham entering the air is subject to rapid indirect photochemical degradation (DT_{50} value of 10.8 hours for hydroxyl radical reaction).

Ready Biodegradability

A ready biodegradability study was available in the RAR. The test followed OECD TG 301D. 21 % of Desmedipham was degraded after 28 days. As the degradation of the substance is lower than the trigger value of 60 % within 28 days for respirometric methods, Desmedipham is not considered readily biodegradable.

Aerobic mineralisation in surface water

Aerobic mineralisation of [aniline-UL-14C]-Desmedipham in surface water was investigated according to OECD TG 309. The radiolabelled test item was applied in water at concentrations of 0.1 and 0.01 mg/L. Additionally, the high concentration experiment was performed under sterile conditions in order to gain information about abiotic degradability of the test item. The pH ranged from 7.61 to 8.44 for all test systems treated with Desmedipham.

Desmedipham dissipated rapidly in surface water with a half-life of less than one day, regardless of its concentration. The main degradation product, in both the high and low dose system, was aniline. CO_2 formation represented around 5 %. The calculated half-lives, single first order (SFO), for the dissipation of Desmedipham were 0.004 days in the high concentration test systems and 0.12 days in low concentration test systems. The half-lives of the degradation product aniline were 75.9 days and 34.9 days for high and low concentrations, respectively. The half-life of diphenyl urea could only be estimated for high concentration test systems, with a value of 2.7 days.

Water-sediment

Six studies on the route and rate of degradation of [aniline-UL-14C]- and [phenoxy-ring-UL-14C]-labelled Desmedipham in water/sediment systems were considered valid in the RAR. The studies followed the OECD TG 308. The results are based on the worst-case outcomes of kinetic analyses.

The estimated half-lives of Desmedipham ranged from 0.035 to 3.1 days in total system and from 0.024 to 4 days in water phase. Mineralisation rate varied among studies and different water sediment systems from 56-66.4 % at a pH = 8.2 in the study **RAR B.8.2.2.3/05, 1994 & B.8.2.2.3/06** to 14.1-43.7 % at pH 6.1 and 7.3 respectively in the study **RAR B.8.2.2.3/07, 2003 & B.8.2.2.3/08, 2003**.

The estimated half-lives in total system of degradants aniline, EHPC and phenol ranged from 0.23 to 47.1 days, 6.4 to 64.2 days and 0.3 to 4.3 days, respectively. Based on the results, the primary degradation of Desmedipham and the degradant phenol will be rapid in natural environments. The degradants aniline and EHPC, however, were less degradable.

Soil degradation data

Eight studies (four with Desmediphan and the others with degradation products) of degradation in soil under aerobic conditions were considered valid in the RAR. The studies were performed according OECD TG 307.

The estimated half-lives of Desmedipham in soil ranged from 3.7 to 127.2 days.

Conclusion on degradation

Overall, degradation information does not provide sufficient data to show that Desmedipham is ultimately degraded to above 70 % within 28 days (equivalent to a half-life of less than 16 days) or being transformed to non-classifiable products. Therefore, Desmedipham is considered being not rapidly degradable, according to the CLP criteria.

Bioaccumulation

Three bioaccumulation studies and one re-evaluation report were included in the RAR.

In the study **RAR 8.2.2.3/02, 1994 & B.9.2.2.3/04, 1993** the bioaccumulation of Desmedipham was tested with 75 fish (*Oncorhynchus mykiss*; Rainbow trout) in 6.2 µg/L group, 73 fish in 62 µg/L group and 25 fish in control group. The fish were exposed for 7 days in a flow-through system followed by a 14-day depuration period.

The study met the validity criteria for the updated OECD TG 305. In the test, Desmedipham concentrations varied between 47.1 and 78.3 % in the lower dose level and between 46.5 and 79.9 % in the higher dose level.

BCFs values were in the range of 317.7-333.9 L/kg in whole fish. No growth correction or lipid normalisation was applied.

In 2 studies (RAR **B.9.2.2.3/01& B.9.2.2.3/04),** the active substance was almost totally hydrolysed in the study and the results mainly represent the bioaccumulation potential of the two degradation products, thus, the BCF value could not be calculated based on Desmedipham only.

In RAR **B.9.2.2.3/03**, the bioaccumulation of Desmedipham was tested with 85 fish (rainbow trout) at nominal test concentrations of 100 and 500 µg/L. The study was generally in line with a previous version of OECD TG 305 (1996). Yet, some of the validity criteria for the updated OECD TG 305 (2012) were met as well. According to OECD TG 305, valid results can only be obtained with stable substances, so bioconcentration could only be determined when based on the first 48 hours of the test and the depuration phase. Results were similar at both nominal concentrations of 100 and 500 µg/L, with BCF_{SS} values 64 and 65 L/kg, respectively. In order to assess total bioconcentration of degradation products, BCF_{SS} value of 72 was calculated for the total radioactivity in fish.

The log P_{OW} for Desmedipham and its degradants were estimated by conducting tests according to OECD TGs 107 and 117 (RAR B.2.7/01, 2004-2016). The study, considered valid, resulted in log K_{OW} value of 3.5. Desmedipham degradation products EHPC (log P_{OW} = 0.87), aniline (log P_{OW} = 0.9), phenol (log P_{OW} = 1.47) and diphenyl urea (log P_{OW} = 2.3) are not considered bioaccumulative as their log P_{OW} does not exceed 4.

Conclusion on bioaccumulation

The log K_{OW} value for Desmedipham (3.5) and for the degradation products aniline (0.9), EHPC (0.87), phenyl (1.47) and diphenyl urea (2.3) were measured according to OECD TGs 107 and

117. In the experimental studies following OECD TG 305, the highest BCF_{SS} values in whole fish for Desmedipham only was 65 L/kg and for Desmedipham and the degradants was 333.9 L/kg (as determined from ^{14}C-labelled compounds). Based on these values the substance has a low potential to bioaccumulate.

Aquatic Toxicity

The following tables summarise acute and chronic toxicity studies considered in the CLH report:

Method	Species	Test material	Results mg/L	Remarks	Reference
Acute toxicity to fish - Desmedipham					
OECD TG 203 GLP	*Cyprinus carpio* (common carp)	Desmedipham technical Purity 98.2 % w/w	96h LC_{50} 4.83 (mm)	Fulfilled the validity criteria	2004, 2017 M-232623-01-1 M-594667-01-1 dRAR B.9.2.1/06
OECD TG 203 GLP	*Oncorhynchus mykiss* (rainbow trout)	Desmedipham technical Purity 98.9 % w/w	**LC_{50} 1.41 mg a.s./L** **Key study**	Fulfilled the validity criteria	2016 M-564890-01-1 dRAR B.9.2.1/14
Acute toxicity to *Daphnia magna* - Desmedipham					
OECD TG 202; USEPA 72-2; US EPA OCSOO: 850.1010 GLP	*Daphnia magna* (cladoceran)	Desmedipham technical Purity 96.8 % w/w	48h EC_{50} 0.78 (nom) **0.35 mg/L** (mm) **Key study**	Fulfilled the validity criteria	1996, 2016 M-146483-01-1 M-545523-01-1 dRAR B.9.2.4.1/03
OECD TG 202; U.S. EPA OPPTS Nr. 850.1010, GLP	*Daphnia magna* (cladoceran)	Desmedipham technical Purity 99.5 % w/w	48h EC_{50} > 1.1 (mm of sum of parent and metabolite) EC_{50} > 0.33 mg/L (mm)	Fulfilled the validity criteria	2012 M-438144-02-1 dRAR B.9.2.4.1/05
ISO 6341, the EEC directive 92/69, Part C.2.; OECD TG 202	*Daphnia magna* (cladoceran)	EHPC (ethyl-3-hydroxyphenylcarbamate Purity > 99 % w/w	48h EC_{50} 22 (nom)	Fulfilled the validity criteria	1998 M-494070-01-1 dRAR B.9.2.4.1/07
Acute toxicity to Americamysis bahia - Desmedipham					

OPTTS Guideline 850.1035 GLP	*Americamysis bahia* (mysid shrimp)	Desmedipham technical Purity 99.5 % w/w	96h LC_{50} 1.2 (mm sum of Desmedipham and EHPC) 96h LC_{50} 0.49 (mm)	Fulfilled the validity criteria	2011 M-409869-01-1 dRAR B.9.2.4.2/01
Acute toxicity to green algae - Desmedipham					
OECD TG 201 GLP	***Selenastrum capricornutum (green algae)***	Desmedipham technical Purity 96.8 % w/w	**96h E_rC_{50} ~ 0.064** (mm) 24h E_rC_{50} 0.059 48h E_rC_{50} 0.097 72h E_rC_{50} 0.228 (im) **Key study**	Fulfilled the validity criteria	1993, 2004 M-146929-02-1 M-146929-02 dRAR B.9.2.6.1/02
Toxicity to aquatic macrophytes – *Lemna gibba* - Desmedipham					
OECD TG 221; US EPA: 123-2; GLP	*Lemna gibba* (duck weed)	Desmedipham Purity 98.2 % w/w	E_rC_{50} > 5.2 mg/L (im) E_rC_{50} > 0.229 mg/L (geometric mm)	Validity criteria met	2002, 2016 M-241092-01-1 M-545827-01-1 dRAR B.9.2.7/02
OECD TG 221, GLP	*Lemna minor* (duck weed)	Desmedipham technical Purity 97.4 ± 0.22 %	7d-E_rC_{50} 0.85 mg/L (nom) 7d-E_rC_{50} 0.40 mg/L (geometric mm)	Fulfilled the validity criteria	2004, 2016 M-494089-01-1 M-545827-01-1 dRAR B.9.2.7/03
OECD TG 221, GLP	*Lemna gibba* (duck weed)	Desmedipham technical Purity 99.5 % w/w	7d- E_rC_{50} 8.41 mg/L (nom) **7-d E_rC_{50} 0.113 mg/L** (geometric mm**)** **Key study**	Fulfilled the validity criteria	2012, 2016, 2017 M-444430-01-1 M-545827-01-1 M-594283-01-1 dRAR B.9.2.7/04
Toxicity to aquatic macrophytes – *Myriophyllum spicatum* - Desmedipham					
OECD TG	*Myriophyllu*	Desmedipham technical	14-d E_rC_{50}	Validity	2013, 2016

221, GLP	*m spicatum* (Eurasian watermilfoil)	Purity 99.5 % w/w	> 5.0 mg/L (nom) 14-d E_rC_{50} > 0.05 mg/L (geometri c mm)	criteria met	M-461454-01-1 M-545827-01-1 dRAR B.9.2.7/08

Method	Species	Test material	Results[1]	Remarks	Reference
Chronic toxicity to fish - Desmedipham					
EU Directive 91/414 EEC; Regulation (EC) No. 1107/2009 US EPA OCPP 850.1400 GLP	*Oncorhynchus mykiss* (rainbow trout)	Desmedipham technical Purity 99.5 % w/w	**EC_{10} 0.146 mg/L** (arithmetic mm) **key study**	Fulfilled the validity criteria	2014, 2016 M-482005-01-1 M-545521-01-1 M-582216-01-1 dRAR B.9.2.2.1/01
Chronic toxicity to Daphnids - Desmedipham					
OECD TG 211 U.S.EPA OPPTS 850.1300, GLP	*Daphnia magna* (cladoceran)	Desmedipham technical Purity 99.5 % w/w	NOEC 0.049 mg/L (mm of sum of Desmedipham and EHPC) **NOEC 0.020** mg/L (arithmetic mm) **key study**	Fulfilled the validity criteria	2012 M-437659-02-1 dRAR B.9.2.5.1/03
Chronic toxicity to aquatic macrophytes – *Lemna minor* – Desmedipham					
OECD TG 221, GLP	*Lemna minor* (duck weed)	Desmedipham technical Purity 97.4 ± 0.22 %	7 d NOEC 0.0492 mg/L (geometric mm)	Fulfilled the validity criteria	2004, 2016 M-494089-01-1 M-545827-01-1 dRAR B.9.2.7/03
Chronic toxicity to aquatic macrophytes – *Lemna gibba* – Desmedipham					
OECD TG 221 GLP	*Lemna gibba* (duck weed)	Desmedipham technical Purity 99.5 % w/w	E_rC_{10} 0.013 mg/L (geometric mm) E_rC_{10} 0.011 mg/L (geometric	Fulfilled the validity criteria	2012, 2016, 2017 M-444430-01-1 M-545827-01-1 M-594283-01-1 dRAR

			mm)		B.9.2.7/04
Toxicity to aquatic macrophytes – *Myriophyllum spicatum* – Desmedipham					
OECD TG 221 GLP	***Myriophyllum spicatum* (Eurasian watermilfoil)**	Desmedipham technical Purity 99.5 % w/w	**14 d NOE_rC 0.002 mg a.s/L** (geometric mm) **Key study**	Fulfilled the validity criteria	2013 M-461454-01-1 dRAR B.9.2.7/08

[1]mm – mean measured concentrations; im – initial measured concentrations; non – nominal concentrations

In the CLH report, there are also studies available with the degradation products but none of the available studies on the transformation products indicate a higher toxicity than the parent and hence the studies have not been included in the table above or the summaries below.

Acute toxicity to Fish

Two acute toxicity tests with Desmedipham were considered valid in the RAR. The lowest 96h LC_{50} value of 1.41 mg/L was determined with *Oncorhynchus mykiss* based on mean measured concentrations **(RAR B.9.2.1/14)**. In the test acute toxicity of Desmedipham to rainbow trout was studied over 96h in a semi-static test according to OECD TG 203 and in compliance with GLP. Nominal test concentrations were 0.250, 0.500, 1.00, 2.00, 4.00 mg a.s./L. Mean measured concentrations were 0.233, 0.475, 0.999, 2.06 and 4.15 mg a.s./L.

Acute toxicity to Invertebrates

Two valid acute toxicity studies with Desmedipham on *Daphnia magna* were available. In addition, the toxicity of Desmedipham to *Americamysis bahia* was also studied. The lowest endpoint with Daphnia magna is EC_{50} = 0.35 mg/L.

In test **RAR B.9.2.4.1/03** the acute toxicity of Desmedipham to *Daphnia magna* was studied in a 48h flow-through test according the OECD TG 202; US EPA: 72-2 OCSOO: 850.1010 and in compliance with GLP. Twenty daphnids per concentration, divided into 2 groups of 10, were exposed to nominal concentrations of 0, 0 (solvent control), 0.41, 0.69, 1.2, 1.9 and 3.2 mg/L. The arithmetic mean measured concentrations of Desmedipham were 0, 0 (solvent control), 0.13, 0.32, 0.57, 0.93 and 1.6 mg a.s./L. Desmedipham and EHPC were measured from test water at the beginning (0h) and at the end of the test (48h) by HPLC. The 48h EC_{50} value based on arithmetic mean measured concentration of Desmedipham was 0.35 mg a.s./L.

Chronic toxicity to fish

One valid chronic test with Desmedipham on fish *Oncorhynchus mykiss* is available in the RAR. Test results from two prolonged fish test were also presented in the RAR but not evaluated, as early-life stage toxicity test is preferred for evaluating chronic hazard. Those results were not presented either in the CLH report and only studies which are relevant for classification are summarised below.

In **RAR B.9.2.2.1 /01**, the chronic toxicity of Desmedipham to rainbow trout was studied in an early life stage test according to guideline US EPA OCSPP 850.1400. Rainbow trout (starting with eggs less than 24 hours old) were exposed to Desmedipham in a flow-through system over a period of 92 days. Nominal concentrations were 0.0667, 0.120, 0.216, 0.389 and 0.700 mg a.s./L. The test concentrations based on arithmetic mean measured concentrations of

Desmedipham were 0.076, 0.115, 0.197, 0.405 and 0.683 mg a.s./L, respectively. The test resulted in an EC_{10} value of 0.146 mg a.s./L based on mean measured concentrations.

Chronic toxicity to Invertebrates

In total, three aquatic invertebrate studies with Desmedipham were included in the Dossier. One long-term toxicity test for *Daphnia magna* **(RAR B.9.2.5.1/03)** represented the lowest endpoint NOEC = 0.02mg/L, as well as two other tests of Desmedipham with *Chironomus riparius* **(RAR B.9.2.5.3/01; RAR B.9.2.5.3/02**).

In test **RAR B.9.2.5.1/03,** chronic toxicity of Desmedipham to *Daphnia magna* was studied during 21 days in a flow-through test according to OECD TG 211; USEPA (=EPA): OPPTS 850.1300, where less than 24 daphnids were exposed to nominal concentrations of 6.3, 13, 25, 50 and 100 µg a.s./L. The arithmetic mean measured concentrations of Desmedipham were 4.1, 6.3, 10, 20 and 38 µg a.s./L.

Observations of the effects of Desmedipham on survival, reproduction and growth were used to determine endpoints. The EC_{10} and EC_{20} for dry weight were higher than 38 µg/L. Regarding the number of offspring, immobility of adults and length at the end of the study, effects were observed at the highest concentration only. Consequently, no significant dose-response relationship is observed and no valid EC_{10} or EC_{20} values can be calculated for these endpoints. The NOEC for growth (based on length), survival and reproduction was 0.02 mg/L a.s. and the LOEC was 38 µg a.s./L based on mean measured test concentrations.

In addition, in test **RAR B.9.2.5.3/01**, the chronic toxicity of Desmedipham to *Chironomus riparius* was studied during 28 days under a static test condition according to US EPA OPPTS 850.1735. The test fulfilled validity criteria. Based on initial Desmedipham concentration a NOEC value of 1.0 mg/L was obtained. NOEC = 0.14 mg a.s./L based on measured concentrations.

In the sediment toxicity study **(RAR B.9.2.5.3/02)**, groups of 20 midges of *Chironomus riparius* in four replicates were exposed to five test concentrations of Desmedipham. A NOEC based on an initially measured concentration of 3.34 mg a.s./L was obtained. The test was done according to Draft OECD 219 and fulfilled validity criteria.

Algae and aquatic plants

Only one toxicity study with Desmedipham on green algae was considered valid in the RAR (**RAR B.9.2.6.1/02**). In addition, studies with aquatic macrophytes *Lemna gibba* (**RAR B.9.2.7/02; RAR B.9.2.7/04**) (duck weed), *Lemna minor* RAR B.9.2.7/03 and *Myriophyllum spicatum* (Eurasian watermilfoil) (**RAR B.9.2.7/08**) were presented.

In the study **RAR B.9.2.6.1/02,** the toxicity of Desmedipham to *Selenastrum capricornutum* was studied according to OECD TG 201. Triplicate algal cultures with a cell count of approximately 1×10^4 cells/mL were exposed for 96h at nominal concentrations (i.e. 0.065, 0.11. 0.18, 0.3 and 0.5 mg/L). The initial measured concentrations of Desmedipham were: 0.053, 0.084, 0.141, 0.178, and 0.619 mg/L. Desmedipham was found on day 3 only in the two highest treatment levels in concentrations of 7 % (0.013 mg/L) and 3.2 % (0.020 mg/L) of initial, respectively. No Desmedipham could be detected in any of the test treatments on day 4 due to degradation, principally by hydrolysis, and therefore results were based on initial measured concentrations. The solvent control data was used for all calculations.

The 72h E_rC_{50} was 0.228 mg/L, 48h E_rC_{50} was 0.097g/L and 24h E_rC_{50} was 0.059 mg/L based on initial measured concentrations. The NOEC was less than 0.05 mg/L. In the test report, it was suggested to use E_rC_{50} value either for 24h 0.059 mg/L or 48h 0.097 mg/L. This was based

on the observed substantial increase in cell growth after 48 hours of the test and when the pH began to increase, indicating recovery of the algal cultures as Desmedipham degraded.

In addition, in the RAR a 72h E_rC_{50} value of 0.064 mg/L based on geometric mean measured concentrations was reported. The DS suggested using the estimated E_rC_{50} value of 0.064 mg/L for classification. Using the 48h E_rC_{50} value of 0.097 mg/L or 24h E_rC_{50} value of 0.059 (based on the initial measured concentrations) would result the same classification.

The effects of Desmedipham to duckweed *Lemna gibba* was studied over 7 days according to OECD TG 221 and US EPA guidelines **(RAR B.9.2.7/02).** Three replicates of *Lemna gibba* were exposed to nominal concentrations of 0.03, 0.09, 0.25, 0.72, 2.08 and 6.0 mg a.s./L. Initial measured concentrations were 0.02, 0.07, 0.18, 0.52, 1.71, 5.2 mg/L. Measured concentrations were 0.0148, 0.0261, 0.0425, 0.0729, 0.1312, 0.2286 mg/L. 7d E_rC_{50} and E_bC_{50} values were > 5.2 mg/L based on mean measured initial concentrations and E_rC_{50} >0.229 mg a.s./L based on mean measured concentration. The 7d NOEC values for growth rate, biomass and frond dry weight were 0.52 mg/L based on initial concentrations or 0.079 mg/L based on measured concentration.

The toxicity of Desmedipham on *Lemna minor* was studied in a 14d test according to OECD TG 221 **(RAR B.9.2.7/03)**. *Lemna minor* was exposed in three replicates to nominal concentration levels 0.032, 0.1, 0.32, 1.0 and 3.2 mg a.s./L. Geomean concentrations were 0.0145, 0.0492, 0.1533, 0.4621, 1.5623 mg a.s./L. The 7 day E_rC_{50} value was 0.85 mg a.s./L based on nominal concentrations of Desmedipham and 0.50 mg a.s./L and 0.40 mg a.s./L based on arithmetic and geometric mean measured concentrations of Desmedipham, respectively. The 7 day NOE_rC was 0.0492 mg a.s./L based on geomean measured concentrations.

In the test **RAR B.9.2.7/04,** *Lemna gibba* fronds were exposed to Desmedipham for 7 days to the nominal concentrations of 0.0780, 0.156, 0.313, 0.625, 1.25, 2.50, 5.00 and 10.0 mg a.s./L in according to OECD TG 221. Geometric measured concentrations were 0.0074, 0.0107, 0.0149, 0.0208, 0.0295, 0.0409, 0.0579, 0.165 mg/L. A 7 d E_rC_{50} value of 8.41 mg a.s./L was obtained, based on nominal concentrations, and a 7 d E_rC_{50} value of 0.113 mg a.s./L based on geometric mean measured concentrations of Desmedipham. The NOE_rC value was 0.0107 mg a.s./L based on geometric mean measured concentrations of Desmedipham.

In the test **RAR B.9.2.7/08,** *Myriophyllum spicatum* shoots were exposed via the water phase to the test item for 14 days in a semi-static toxicity test. Nominal concentrations were 0.015, 0.048, 0.15, 0.49, 1.56, 5 mg a.s./L whereas geometric mean measured concentrations were 0.0020, 0.0034, 0.0057, 0.0107, 0.0190, 0.0499 mg/L. In the test, sediment samples were not collected for chemical analysis. The study was performed according to the OECD TG 221, but the study followed in principle the OECD TG 239 except that the replication was smaller than recommended by OECD TG 239. Otherwise the test fulfilled the validity criteria. The 14 day E_rC_{50} value was > 5 mg a.s./L where 29 % inhibition was obtained. This value is based on nominal concentrations of Desmedipham corresponding to >0.05 mg a.s./L based on geometric mean measured concentrations. The 14 day NOE_rC value was 0.002 mg a.s./L based on geometric mean measured concentrations.

Conclusion on acute classification

A full acute data set (fish, aquatic invertebrates, algae and aquatic macrophytes) is available for Desmedipham. The classification proposal by the DS is based on studies conducted with Desmedipham as the lowest and the most reliable endpoint values for classification purpose were obtained with the parent substance.

The lowest EC_{50} value for fish was 1.41 mg/L (*Oncorhynchus mykiss*), for aquatic invertebrates 0.35 mg/L (Daphnia magna) and for aquatic plant 0.113 mg/L (Lemna gibba). 72 h E_rC_{50} value of 0.064 mg/L was estimated for green algae *Selenastrum capricornutum*. Based on the available data it is concluded that Desmedipham does fulfil the criteria for classification as Aquatic Acute Category 1. An M factor of 10 is warranted based on the *Selenastrum capricornutum* 72h E_rC_{50} 0.064 mg/L (M factor 10 when $0.01 < L(E)C_{50} \leq 0.1$).

Conclusion on chronic classification

Desmedipham is considered by the DS to have a low potential to bioaccumulate and is not rapidly degradable. Adequate chronic toxicity data for Desmedipham was available for three trophic levels fish, aquatic invertebrates including sediment dwelling organisms and algae and aquatic plants.

The lowest endpoint values were for fish EC_{10} of 0.146 mg/L (*Oncorhynchus mykiss*), aquatic invertebrate NOEC of 0.020 mg/L (Daphnia magna), and for aquatic macrophyte 14d-NOE_rC of 0.002 mg/L (*Myriophyllum spicatum*) which was the most sensitive species. Based on the available data it is concluded that Desmedipham does fulfil the criteria for classification as Aquatic Chronic Cat. 1. An M factor of 10 is warranted based on the *Myriophyllum spicatum* 14d-NOE_rC of 0.002 mg/L (M factor 10 for non-rapidly degradable substance when $0.001 < NOEC \leq 0.01$).

Comments received during public consultation

Three Member States (MS) commented during public consultation. Two of them agreed with the proposed classification. The third MS commented on the following issues:

Algal growth inhibition study

The MS indicated that statistically derived endpoints using mean measured treatments would be better. It also asked for validity criteria check for the control due to a pH variation higher than the TG recommendation of 1.5 units over the study period.

The DS agreed that it would be useful to run statistical analysis with geometric mean measured concentrations. Unfortunately, they did not have an appropriate statistical program to run such analysis. Regarding the validity of the study the control data was compared to the criteria set in OECD TG 201 (2011) and fulfilled them.

RAC considers the study valid since it fulfils validity criteria. RAC agrees that statistically derived endpoints are preferred and has calculated them based on geometric mean measured concentrations. Results show that 72h endpoints provide a better fit than for 48h. E_rC_{50} = 0.045 mg/L ± 2 × 0.01353855; EC_{10}= 0.014 mg/L ± 2 × 0.00098038 mg/L.

Additional algal growth inhibition studies

In addition, the MS highlighted that additional algal data included in the RAR (2017), but not presented in the CLH due to the lack of 'intermediate' analytical measurements, should be considered if valid.

The DS indicated that there were four other algae studies included in the RAR. Two of them were rejected but two were deemed appropriate. These studies do not change the classification outcome. The DS included in the RCOM a small summary of these studies.

The static test performed in 2005 with *Desmodesmus subspicatus* provided a NOEC value of

1.34 mg/L and an E_rC_{10} = 0.0128 mg/L.

In the other study performed in 2011 with *Pseudokirchneriella subcapitata (*now *Raphidocelis subcapitata)* EC_{50}, EC_{20} and EC_{10} were > 0.032 mg/L, 0.0164 mg/L and 0.0080 mg/L, respectively.

RAC agrees and considers that the lack of intermediate measurements does not invalidate the studies. The tests fulfil validity criteria of OECD TG 201 and are considered valid by RAC.

Myriophyllum spicatum study

The MS indicated that given the rapid loss of the test item it would be more appropriate to determine the geometric mean for each renewal period and calculate the mean exposure over the whole exposure period calculated from this data, although this would result in a NOEC in the same classification range.

The DS responded that the test results were based on geometric mean measured concentration and samples from each renewal period were already taken into account in the calculations.

RAC agrees with the DS response and considers calculations based on geomean measured concentrations appropriate.

Chironomus riparius:

The MS also pointed out that due to the significant and rapid loss of the test item in both studies, 28-day NOECs based on initial measured concentrations may not be appropriate. In this sense, they asked to consider a time-weighted average endpoint.

The DS concluded for test RAR B.9.2.5.3/01 that TWA was not justified since no analytical results are available for day 0.

RAC agrees with the DS response and considers that using TWA would result in unrealistically low effect values in a static test of 28 days duration where the substance disappears so fast. For the second test, RAR B.9.2.5.3/02, the DS indicated that a NOEC = 0.0246 mg a.i./L based on TWA was calculated.

In this test it is not clear for RAC how a NOEC was calculated when in the test it is stated that no treatment related effects were observed. If no statistically related effects were observed at all, the NOEC should be higher than and not equal to the highest concentration tested.

Assessment and comparison with the classification criteria

Degradation

Desmedipham is hydrolytically stable under acidic conditions and one of the degradation products, aniline (CAS 62-53-3), has a harmonised classification as hazardous to the aquatic environment under CLP (Aquatic Acute 1).

In a ready biodegradability test (OECD TG 301D) Desmedipham showed only 21 % of degradation after 28 days and is therefore not readily biodegradable. Degradation information did not provide sufficient data to show that Desmedipham is ultimately degraded to above 70 % within 28 days (equivalent to a half-life of less than 16 days) or being transformed to non-classifiable products. Under neutral and alkaline conditions, Desmedipham undergoes fast primary degradation with a half-life below 16 days, but aniline is a significant transformation product, and has a harmonised classification as hazardous to the aquatic environment.

RAC agrees with the DS to consider Desmedipham as **not rapidly degradable** according to the CLP criteria.

Bioaccumulation

In the experimental studies according to OECD TG 305, the highest BCF_{SS} value in whole fish for Desmedipham only was 65 L/kg and for Desmedipham and 333.9 L/kg for the degradant (as determined from 14C-labelled constituents). The values obtained are lower than the CLP trigger value of 500.

The log K_{OW} value for Desmedipham (3.5) and for the degradation products aniline (0.9), EHPC (0.87), phenyl (1.47) and diphenyl urea (2.3), measured according to OECD TGs 107 and 117, are lower than the CLP log K_{OW} trigger value of ≥ 4.

RAC agrees with the DS to consider Desmedipham as not bioaccumulative for classification and labelling.

Acute aquatic toxicity

A full acute data set is available for Desmedipham as there were reliable acute toxicity studies available for fish, aquatic invertebrates, algae, and aquatic macrophytes. Also, studies with metabolites EHPC, aniline and phenol were available for all trophic levels. The degradant aniline (CAS 62-53-3) has a harmonised classification of Aquatic Acute 1 under CLP. The proposed classification is based on studies conducted with Desmedipham as the lowest and the most reliable endpoint values were obtained from studies with the parent substance.

The lowest endpoints for each trophic level are:

- Fish *Oncorhynchus mykiss* LC_{50} (96h) = 1.41 mg/L
- Invertebrates *Daphnia magna* EC_{50} (48h) = 0.35 mg/L
- Algae *Selenastrum capricornutum* E_rC_{50} (72h) = 0.045mg/L

Desmedipham fulfils the criteria for classification as Aquatic Acute Category 1. An M factor of 10 is warranted based on the *Selenastrum capricornutum* 72h E_rC_{50} M factor 10 ($0.01 < L(E)C_{50} \leq 0.1$).

Chronic aquatic toxicity

There is reliable chronic toxicity data available for Desmedipham for fish, invertebrates, algae, and aquatic plants. The lowest and the most reliable endpoint values for classification purpose were obtained from studies with the parent substance Desmedipham. The lowest endpoints for each trophic level are:

Fish Oncorhynchus mykiss EC_{10} (92d) = 0.146 mg/L

- Invertebrates *Daphnia magna* NOEC (21d) = 0.020 mg/L
- Alga *R. subcapitatus* EC_{10} (72h) = 0.0080 mg/L
- Aquatic macrophyte *Myriophyllum spicatum* 14d-NOE_rC (14d) of 0.002 mg/L

The lowest endpoint corresponds to a water sediment test with *Myriophyllum spicatum,* a rooted Macrophyte. In this test sediment samples were not collected for chemical analysis. However, the mode of action of the active substance, an herbicide that acts via the foliage of emerged weeds and inhibits the Hill-reaction; the fact that the substance was applied into the

water column and the higher sensitivity of this species to Desmedipham chronic exposure justify its use for chronic classification.

Thus based on the available data it is concluded that Desmedipham fulfil the criteria for classification as Aquatic Chronic Cat. 1. An M factor of 10 is warranted based on the *Myriophyllum spicatum* 14d-NOE_rC of 0.002 mg/L (M factor 10 for non-rapidly degradable substance when 0.001 < NOEC ≤ 0.01).

Conclusion on the classification

RAC agrees with the DS that Desmedipham fulfils the CLP criteria for classification as **Aquatic Acute 1; H400 with an M-factor of 10 and Aquatic Chronic 1; H410 with M-factor of 10.**

Supplemental information - In depth analyses by RAC

Algae growth inhibition study with Selenastrum capricornutum RAR B.9.2.6.1/02

In the study, the toxicity of Desmedipham (purity 96.8 %) to *Selenastrum capricornutum* was studied during 96 h in a static test according to OECD TG 201. During PC a comment was made on the preference for dose response statistically derived endpoints.

RAC has attempted to derive statistical endpoints. Since there are lost samples for some concentrations and the concentration goes below the LOD, RAC has calculated geomean concentrations considering initial measured concentrations and LOD/2 where the concentration was not detected, following the CLP Guidance.

The next table shows concentrations measured during the test and geomean concentrations used for $E_rC_{50/10}$ re-calculations.

Table 1*: Nominal, initial measured and geomean concentrations*.

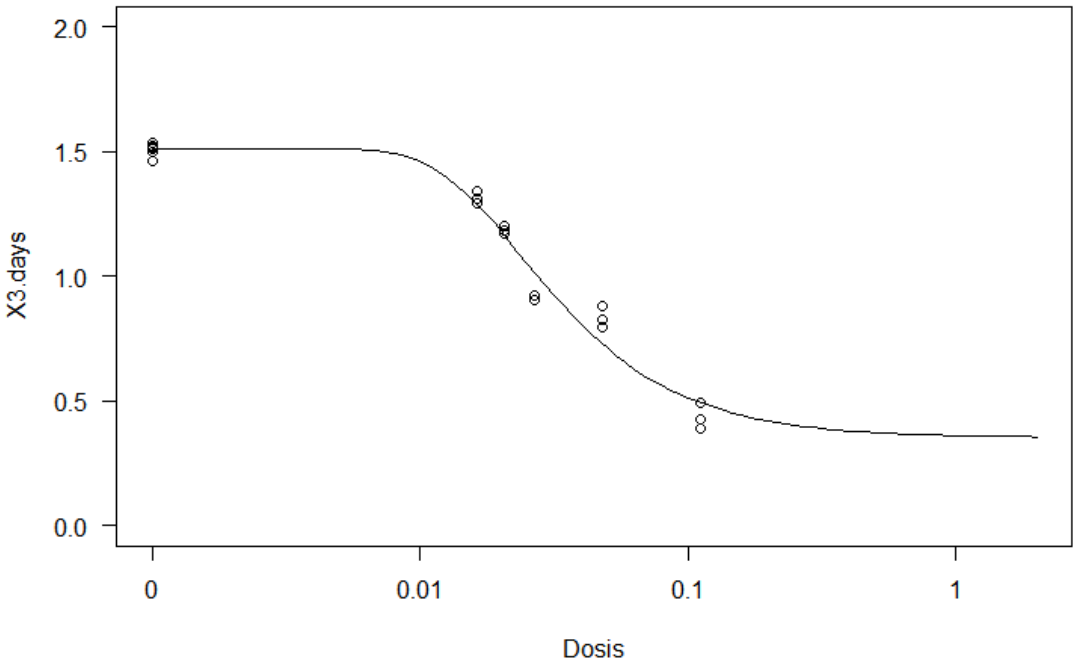
Nominal	Conc (0h)	Conc (24h)	Conc (48h)	Conc (72h)	Geomean 48h	Geomean 72h
0.065	0.053	Lost	0.005	0.005	0.016	0.016
0.108	0.084	0.01	0.005	0.005	0.020	0.020
0.18	0.141	0.021	0.013	0.005	0.043	0.027
0.3	0.178	0.041	0.064	0.013	0.107	0.048
0.5	0.619	Lost	0.041	0.02	0.159	0.111

Where the concentration was not detected LOD/2 was considered as proposed in the CLH.

Endpoints for 72h have been obtained plotting concentrations vs average growth rate using the Weibull equation, which provided a better fit than the logistic equation. Results obtained for 72h are of the same order of magnitude as the values provided by the DS:

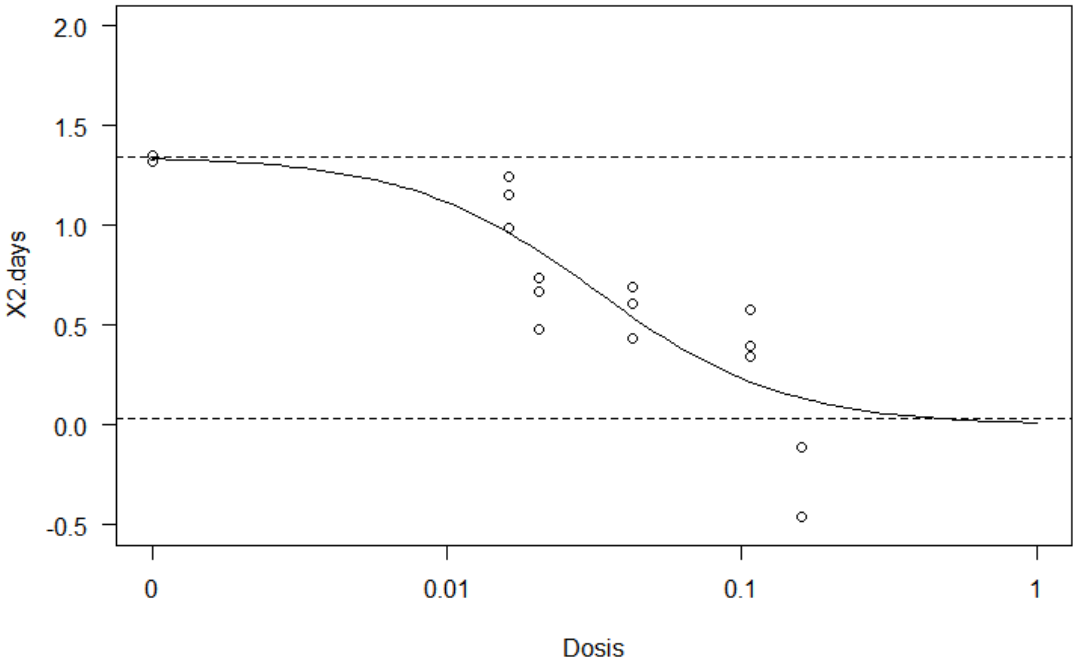
- EC_{10}= 0.014 ± 2 × 0.00098038 mg/L
- EC_{50} = 0.045 ± 2 × 0.01353855 mg/L

Model adjusted for 72h logarithmic scale



For 48h the same approach is followed. In this case, the best fit corresponds to a log logistic model with 3 parameters, although the adjustment is not good:

- EC_{50}= 0.0320138 (0.0206158, 0.0434117)
- EC_{10} = 0.0063979 (0.0012858, 0.0115101)



From the above graphs, it can be seen that data for 72h adjusts better than for 48h, which does not provide a good adjustment. 72h is the preferred endpoint.

The results indicate that with statistically derived endpoints the same classification outcome is obtained. The EC_{50} will be considered to be 0.045mg/L.

Chironomus riparius (RAR B.9.2.5.3/01 and RAR B.9.2.5.3/02)

In these tests exposure via the sediment cannot be ruled out; in addition, the substance has high K_{OC} > 4 000 mL/g) indicating adsorption potential. Since exposure via sediment cannot be discarded, *Chironomus* is a non-target species and CLP is about the hazards in the aquatic environment, the values provided will be used as supporting information. Nevertheless, the endpoints from these tests would not change the classification outcome.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

The hazard class is not assessed in this dossier.

13 ADDITIONAL LABELLING

14 REFERENCES

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15 ANNEXES

Finland, 2017. Revised Renewal Assessment Report (RAR) on desmedipham prepared by the rapporteur MemberState Finland in the framework of Commission Implementing Regulation (EU) No 844/2012, December 2017.