Committee for Risk Assessment

RAC

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

Adopted

20 September 2019

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CLH-O-0000001412-86-294/F

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

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

Chemical name: **desmedipham (ISO); ethyl 3-phenylcarbamoyloxyphenylcarbamate**

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

CAS Number: **13684-56-5; (125579-95-5); (153703-69-6)**

The proposal was submitted by **Finland** and received by RAC on **14 November 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

Finland has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **3 December 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **15 February 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Michal Martínek**

Co-Rapporteur, appointed by RAC: **Ignacio de la Flor Tejero**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **20 September 2019** by **consensus**.

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

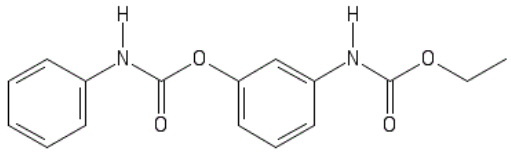
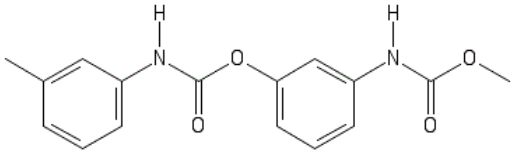
	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	616-113-00-9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

GROUNDS FOR ADOPTION OF THE OPINION

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).

HUMAN HEALTH HAZARD EVALUATION

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 around/below 100 mg/kg bw/d by ca. 10 % MetHb increased to ca. 5 %, Heinz bodies present → Reduction in functional Hb by < 20 %	90-day rat study 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
(4) Reduction in functional Hb at ≥ 20 % due to a combination of Hb reduction and MetHb increase		
(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	None in the mouse; 28-day studies in other species not	28-day mouse study B.6.3.1/01

other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥ 10 %) in a 28-day study	available A possibly "marked" increase in haemosiderosis in the liver in a 90-day dog study, Hb reduction by ca. 10 %	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.**

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 (m/f) 1-year: 10/sex/group 2-year: 60/sex/group	<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 • ↓ 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**.

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 Doses: 0, 10, 100, 500 mg/kg bw/d Dosing GD 6-15 24 females/group	Maternal toxicity ≤ 500 mg/kg bw/d: no adverse effects Developmental toxicity 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	Maternal toxicity 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 %

PNDT studies		
Type of study; Reference; Year	**Method**	**Observations**
	Dosing GD 6-15 25 females/group	≤ 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 B.6.6.2/05 1991	OECD TG 414 GLP Strain: Sprague-Dawley Doses: 0, 60, 250, 1 000 mg/kg bw/d Dosing GD 6-16 25 females/group	<u>Maternal toxicity</u> 1 000 mg/kg bw/d: • ↓ 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

PNDT studies		
Type of study; Reference; Year	**Method**	**Observations**
		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 <u>Developmental toxicity</u> 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

<u>Rat PNDT study B.6.6.2/01</u>

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**.

ENVIRONMENTAL HAZARD EVALUATION

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.	*Daphnia magna*	Desmedipham technical Purity 99.5 % w/w	48h EC_{50} > 1.1 (mm	Fulfilled the	2012 M-438144-0

EPA OPPTS Nr. 850.1010, GLP	(cladoceran)		of sum of parent and metabolite) EC_{50} > 0.33 mg/L (mm)	validity criteria	2-1 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	7d- E_rC_{50} 8.41 mg/L	Fulfilled the	2012, 2016, 2017

		Purity 99.5 % w/w	(nom) **7-d E_rC_{50} 0.113 mg/L** (geometric mm**)** **Key study**	validity criteria	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 221, GLP	*Myriophyllum spicatum* (Eurasian watermilfoil)	Desmedipham technical Purity 99.5 % w/w	14-d E_rC_{50} > 5.0 mg/L (nom) 14-d E_rC_{50} > 0.05 mg/L (geometric mm)	Validity criteria met	2013, 2016 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	Fulfilled the validity criteria	2004, 2016 M-494089-01-1 M-545827-01-1 dRAR

			mm)		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 mm)	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 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.**

ANNEXES:

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

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