Committee for Risk Assessment

RAC

Annex 1

Background document

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

phenmedipham (ISO); methyl 3-(3-methylcarbaniloyloxy)carbanilate

EC Number: 237-199-0
CAS Number: 13684-63-4

CLH-O-0000001412-86-297/F

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

Adopted

20 September 2019

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

phenmedipham (ISO);

methyl 3-(3-methylcarbaniloyloxy)carbanilate

EC Number: **237-199-0**

CAS Number: **13684-63-4**

Index Number: **616-106-00-0**

Contact details for dossier submitter: **Finnish Competent Authority**

Finnish Safety and Chemicals Agency (Tukes)

Finland

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PHENMEDIPHAM (ISO); METHYL 3-(3-METHYLCARBANILOYLOXY)CARBANILATE

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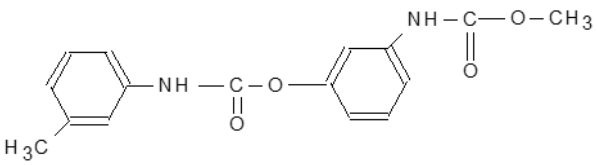
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PHENMEDIPHAM (ISO); METHYL 3-(3-METHYLCARBANILOYLOXY)CARBANILATE

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	methyl 3-(3-methylcarbaniloyloxy)carbanilate; 3-methoxycarbonylaminophenyl 3′-methylcarbanilate
Other names (usual name, trade name, abbreviation)	
ISO common name (if available and appropriate)	phenmedipham
EC number (if available and appropriate)	237-199-0
EC name (if available and appropriate)	phenmedipham
CAS number (if available)	13684-63-4
Other identity code (if available)	77
Molecular formula	$C_{16}H_{16}N_2O_4$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	300.3 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	
Description of the manufacturing process and identity of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	970 g/kg min

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
phenmedipham, CAS 13684-63-4	min. 970 g/kg	Aquatic Acute 1, H400 Aquatic Chronic 1, H410	Aquatic Acute 1, H400 Aquatic Chronic 1, H410

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
toluene, CAS 108-88-3	max 2g/kg	Flam.Liq 2, H225 Skin Irrit. 2, H315 Asp- Tox. 1, H304 STOT SE 3, H336 STOT RE 2, H373 Repr. 2, H361d		No
3-aminophenol, CAS 591-27-5		Acute Tox. 4*, H302 Acute Tox. 4*, H332 Aquatic Chronic 2, H411		No
3-methylaniline, CAS 108-44-1		Acute Tox. 3*, H331 Acute Tox. 3*, H311 Acute Tox. 3*, H301 STOT RE 2, H373 Aquatic Acute 1, H400		No

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

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

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

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

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	616-106-00-0	phenmedipham (ISO); methyl 3-(3-methylcarbaniloyloxy)carbanilate	237-199-0	13684-63-4	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410			
Dossier submitters proposal	616-106-00-0	phenmedipham (ISO); methyl 3-(3-methylcarbaniloyloxy)carbanilate	237-199-0	13684-63-4	**Add** Carc. 2 Repr. 2 STOT RE 2 **Retain** Aquatic Acute 1 Aquatic Chronic 1	**Add** H351 H361d H373 (blood) **Retain** H400 H410	**Add** GHS08 **Retain** GHS09 Wng	**Add** H351 H361d H373 (blood) **Retain** H410		**Add** M = 10 M = 10	
Resulting Annex VI entry if agreed by RAC and COM	616-106-00-0	phenmedipham (ISO); methyl 3-(3-methylcarbaniloyloxy)carbanilate	237-199-0	13684-63-4	Carc. 2 Repr. 2 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H361d H373 (blood) H400 H410	GHS08 GHS09 Wng	H351 H361d H373 (blood) H410		**Add** M = 10 M = 10	

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

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

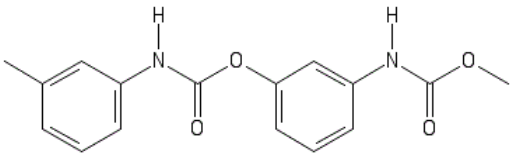
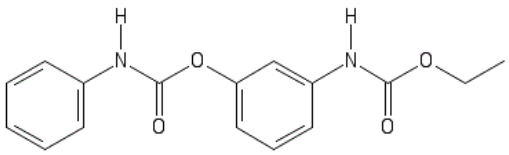
3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

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

RAC general comment

Phenmedipham (ISO); methyl 3-(3-methylcarbaniloyloxy)carbanilate is a herbicide from the phenylcarbamate group.

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

	
phenmedipham	**Desmedipham**

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 absorption, distribution, metabolism, and excretion studies in both RARs). RAC further notes several differences between the toxic effects of phenmedipham and desmedipham: (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 developmental toxicity of phenmedipham to be sufficient upon which to draw a conclusion, it did not see any need to include data on desmedipham 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, October 2017).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

5 IDENTIFIED USES

Phenmedipham is a non-systemic contact herbicide. It acts only via the foliage of emerged weeds. Root uptake is nearly excluded as phenmedipham is strongly absorbed by the soil and is fixed in the upper 5 cm layer. Phenmedipham causes inhibition of the Hill-Reaction in the photosynthetic pathway i.e. it affects the assimilation ability of the plant.

6 DATA SOURCES

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

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Crystalline powder Colourless	1999, 1999a, 1999b, 1999c B.2.3/01 M-193648-01-1, M193657-01-1, M-193651-01-1, M-193653-01-1	
Melting/freezing point	142.7 °C (99.2 % pure) 148.3°C	2012 B.2.1/01 M-440207-01-1	
Boiling point	No boiling point, decomposition begins at 147 °C.	2001 B.2.1/02 M-201044-01-1	The test substance showed an endothermic effect (melting) in the temperature range 140 - 200 °C. An exothermal effect was observed in the temperature range 240 - 390 °C with a maximum energy of 344 J/g. The test substance decomposed before reaching the boiling point, so a boiling point at atmospheric pressure does not exist.
Relative density	Relative density at 20°C compared to water at 4 °C: $D^{20}_4 = 1.31$	2012 M-439467-01-1 B.2.14.	

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Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Vapour pressure	1.6 (± 0.8) x 10^{-7} Pa at 40 °C 6.6 (± 1.1) x 10^{-6} Pa at 51 °C	1992 B 2.2 /01 M-146188-01-1	From this data the extrapolated value was estimated to be 7 x 10^{-10} Pa at 25 °C
Surface tension	The surface tension of the test substance in buffered solution, pH 4 (c = 0.9 mg/L): 70.5 mN/m, at 20 °C ± 0.5 °C. Surface tension of blank buffer solution, pH 4: 70.9 mN/m at 20 ± 0.5 °C.	2012c B.2.12/01 M-439468-01-1	The solubility of the test item in distilled water is below 1 g/L. Therefore, some of the saturated eluate fractions from the water solubility study were diluted to 90% of the saturation solubility (0.9 mg/L). Due to the lack of stability of the test item in solutions with pH values > pH 4, the test was not performed in distilled water but in buffer of pH 4.
Water solubility	pH 3,4: 1,8 mg/l at 20 °C (99.0 % pure) Phenmedipham decomposes at neutral or basic pH	2012 B.2.5/01 M-439488-01-1	1.1 mg/L at 20 °C (buffer pH 4.0, average pH value of saturated solutions 4.1). Due to the lack of stability of the test item in solutions with pH > 4, the solubility of the test item was not determined in distilled water but in buffer of pH 4.0.
Partition coefficient n-octanol/water	$\log P_{o/w}$ = 3.59 at 22 °C (pH = 4) $\log P_{o/w}$ = 2.7 at 20 ± 1 °C (pH = 4) $P_{o/w}$ = 5.0 x 10^2 at 20 °C (pH = 4)	2012b B.2.7/01 M-439458-01-1	Not acceptable. According to COMMISSION REGULATION (EU) No 283/2013 it is now a requirement to include validation data on the methods used in water, buffer solutions, organic solvents and any additional matrices used in the physical and chemical properties tests. Here no validation data has been presented. As 3 is a limiting value of $\log P_{o/w}$ that triggers the demand for some modelling and experiments, the notifier was asked for justification why the end point value 3.59 given in the review report should not be valid. **Answer from Task Force Phenmedipham:** The original study that was used for the inclusion of phenmedipham on Annex I (determined by the shake flask method) was not performed

Property	Value	Reference	Comment (e.g. measured or estimated)
			under GLP and no validation data were presented; therefore a new study was performed using the HPLC-method, taking the expected high $logP_{o/w}$ and the instability of phenmedipham into account. In some individual cases such high differences between the HPLC and shake flask method are possible. The guideline (OECD 117) states: "The inter-laboratory comparison test has shown that with the HPLC method $logP_{o/w}$ values can be obtained to within +/- 0.5 units of the Shake-Flask values".
Flash point			Not required as the melting point of the active substance is higher than 40°C.
Flammability	Not flammable	1995 B.2.9/01 M-145295-01-1	
Explosive properties	Not to be considered as explosive No explosive properties.	2011 B.2.11/01 M-420189-01-1	The heat of decomposition in the three DSC-measurements was below 500 J/g. Therefore, the test on explosive properties was not necessary.
Self-ignition temperature	No self-ignition was registered up to a temperature of 148 °C (melting point of the active substance) . The temperature was not raised to 400 °C due to the low melting point of the substance.	1995 B.2.9/02 M-145295-01-1	
Oxidising properties	No oxidizing properties	1995 B.2.13/01 M-145295-01-1	
Granulometry	-		
Stability in organic solvents and identity of relevant degradation products	-		
Dissociation constant	-		Phenmedipham does not dissociate. No dissociation found in the pH range of 2 < pH < 6. At pH values above

Property	Value	Reference	Comment (e.g. measured or estimated)
			pH 7 a rapid hydrolysis of the substance takes place.
Viscosity	-		

8 EVALUATION OF PHYSICAL HAZARDS

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Absorption from the GI tract

Absorption from the GI tract was studied at three different concentration ranges: 2, 20 and 1000 mg/kg bw. At around 2 mg/kg bw, 80-85% of the administered dose was absorbed within 24 h (single and repeat-dose). At 20 mg/kg bw, using phenyl labelled test substance, the absorbed portion was 50 – 60% within 30 h (1994, RAR B.6.1.1/01), and 70% within 24 h (1989, B.6.1.1/02). The absorption of methylphenyl label in the study B.6.1.1/02 was 55 – 60% within 24 h. At single doses of 1000 mg/kg bw, 9 – 13% of both labels was absorbed within 96 h. Repeated dosage at 20 mg/kg bw during 14 days led to a similar absorption pattern as with single dose administration: about 40-50% of the dose was absorbed within 24-30 h regardless of label (1994, RAR B.6.1.1/01). To conclude, fairly low oral absorption (about 10%) was noted at high doses (1000 mg/kg bw) within 24 h, whereas at mid (20 mg/kg bw) and low (2 mg/kg bw) doses, 50 – 85% of the administered dose was absorbed within 24 h. The oral absorption is thus likely to be fairly low (<80%) at doses exceeding 20 mg/kg bw/day.

Distribution

The distribution of the phenyl label at 20 mg/kg dosage was highest in the GI tract (1.7%) and the liver (0.02%), but detectable levels were also found in kidneys and lungs (1994, RAR B.6.1.1/01). At 1000 mg/kg, less than 1% was found in the GI tract and the liver with both labels; the highest radioactivity was found in plasma, whole blood, lungs, ovaries, thyroid gland, skin, pituitary, heart, adrenal glands, kidneys, spleen and liver. Repeated dosage with 20 mg/kg exhibited somewhat higher residue levels in the GI tract + tissues in males treated with methylphenyl label (2.5%) than with phenyl label (1.3%). Residues in GI tract + tissues were equal (3.4% of the dose) in females treated with the different labels. Retention of residues in liver was 5-10 times more efficient with the methylphenyl label at repeated dosage (max. 0.10%), and higher amounts were also noted in lungs and kidneys of methylphenyl treated rats, as compared to phenyl treated animals. The highest concentrations of radioactivity from the methylphenyl label were found in plasma and whole blood; fairly high levels were found in the thyroid gland, lungs, pituitary, ovaries, kidneys, heart, skin, adrenal glands and spleen. Methylphenyl label was also found in the testes. No indications of accumulation of phenmedipham were noted, but the distribution of radiolabel (especially methylphenyl label) was more efficient at repeated treatment. In the study B.6.1.1/02 (1989) it was also noted much higher retention of total residues of methylphenyl label at 20 mg/kg (15 – 17 mg equivalents phenmedipham/kg tissue), as compared to the phenyl label (0.07 - 0.17 mg equivalents phenmedipham/kg tissue). Highest amounts of methylphenyl label were found in blood and plasma (2-3 mg equivalents phenmedipham/kg tissue), but residues were also found in lungs and thyroids (ca. 1 mg equivalents phenmedipham/kg tissue) in both sexes, and ovaries and kidneys of females (ca. 1 mg equivalents phenmedipham/kg tissue).

Metabolism

Phenyl label:

Both studies (1994, RAR B.6.1.1/01; 1989, B.6.1.1/02) showed that, after enzymatic hydrolysis, methyl-N-(3-hydroxyphenyl) carbamate (MHPC) was the major metabolite in urine of phenyl labelled phenmedipham (20 mg/kg), accounting for 26 – 48% of the administered dose (1994, RAR B.6.1.1/01). The study B.6.1.1/02shows that over 90% of the phenyl label in the urine sample consists of MHPC, the estimated portion of the administered dose being about 60%, in other words somewhat higher than with B RAR B.6.1.1/01. MHPC was found to be excreted mainly as glucuronide and sulphate conjugates. Small amounts of 3-aminophenol (3AP) (up to 6%) and 3-acetamidophenol (3AAP) (up to 10%) were found at the 20 mg/kg phenyl label administration. At 1000 mg/kg administration of phenyl labelled phenmedipham, about 3% MHPC, 0.4% 3AP and 3% 3AAP were determined. No parent compound was detected in urine. In feces, the major component was unchanged phenmedipham (26 – 42% of the dose at 20 mg/kg and 66 – 84% at 1000 mg/kg), suggesting that the dose was mostly unabsorbed. Small amounts of MHPC (less than 1% at 20 mg/kg, and 0.4 – 3.0% at 1000 mg/kg) were observed in feces, as were three other uncharacterised compounds which accounted for less than 5% of the dose. Results from repeated administration of phenyl labelled phenmedipham at 20 mg/kg showed similar metabolic patterns in both urine and feces.

Methylphenyl label:

No single dose studies with the methylphenyl label at 20 mg/kg were performed in the study RAR B.6.1.1/01 (1994). The study B.6.1.1/02(1989), using 20 mg/kg methylphenyl labelled phenmedipham, revealed six metabolites. The major metabolites were 4-acetamido-o-cresol (4AAC) (3 – 42% of the total activity in the urine sample), 3-acetamidobenzoic acid (3AAB) (23 – 48%) and 3-acetamidotoluene (MAT) (4 – 28%). Other identified metabolites were: 5-acetamidosalicylic acid (4HAAB), 3-aminobenzoic acid (3AB) and 4-amino-o-cresol (4AC). In the study RAR B.6.1.1/01 (1994), at a single dose of 1000 mg/kg and at repeated 20 mg/kg doses, confirmed also the existence of 3-aminotoluene (TOL), which is formed as a first step in the metabolic route of the methylphenyl label. At the single high dose and the repeated low dose, 3AAB (1000 mg/kg: 0.1 – 2.7% and 20 mg/kg: 4 – 11%), and 4HAAB (1000 mg/kg: 0.4 – 1.8% and 20 mg/kg: 3 – 8%) were the identified major metabolites. No unchanged phenmedipham was found in urine. The major component in feces was the parent compound. At 1000 mg/kg up to 86% of unchanged phenmedipham was found, and up to 29% in the 20 mg/kg repeated dose study. From the seven minor metabolic fractions observed in feces, constituting 2% of the dose in the high dose study and 5% in the repeat dose study, only MAT could be positively identified.

The proposed metabolic pathway of phenmedipham in rat is shown in Figure 1.

Excretion

Urinary excretion of the phenyl label at 20 mg/kg in the study RAR B.6.1.1/01 (1994) was 50 – 55% within 30 h, and 10 – 13% at 1000 mg/kg within 96 h. Of the methylphenyl label, 8 – 12% was excreted in urine within 96 h at 1000 mg/kg. Fecal elimination of phenyl label was about 40% at 20 mg/kg, and 80 – 85% and 88 – 92% at 1000 mg/kg for the phenyl and methylphenyl labels, respectively. A higher renal excretion of the phenyl label was observed in the study B.6.1.1/02 (1989) with a single dose of 20 mg/kg; about 70% of the label was excreted in urine, whereas a lower renal excretion rate was observed for the methylphenyl label (50 – 55%). Fecal excretion of phenyl label was low (12%) in the study by B.6.1.1/02 (1989), with a somewhat higher excretion rate for the methylphenyl label (28%). Renal excretion at a low dose, 1.9 mg/kg (1971, RAR B.6.1.1/03) of m-aminophenyl (phenyl) labelled phenmedipham was 80 – 85% in 24 h, and fecal excretion accounted only for 5 – 6% of the dose. To conclude on excretion, it may be observed that the proportions of urinary/fecal excretion at 20 mg/kg in two different studies with phenyl label are not in line with each other, but in both cases more than 95% of the administered doses were excreted fairly rapidly, within 24 h, regardless of the route of excretion. Even at 1000 mg/kg, most of the labelled substances (>95%) were excreted within 48 h. Biliary excretion was examined only in one very briefly described study in rats (1969, RAR B.6.1.1/05). The results indicated moderate (12% at 2 h and 0.3% at 8 h; 32% of dose in total in 24 h) biliary excretion for phenyl labelled phenmedipham, whereas lower biliary excretion (2.6% at 2 h and 3.7-6% at 12-24 h; 19% of dose in total in 24 h) was observed with the methylphenyl label. The administered doses were approximately 1.7 – 2.5 mg/kg bw.

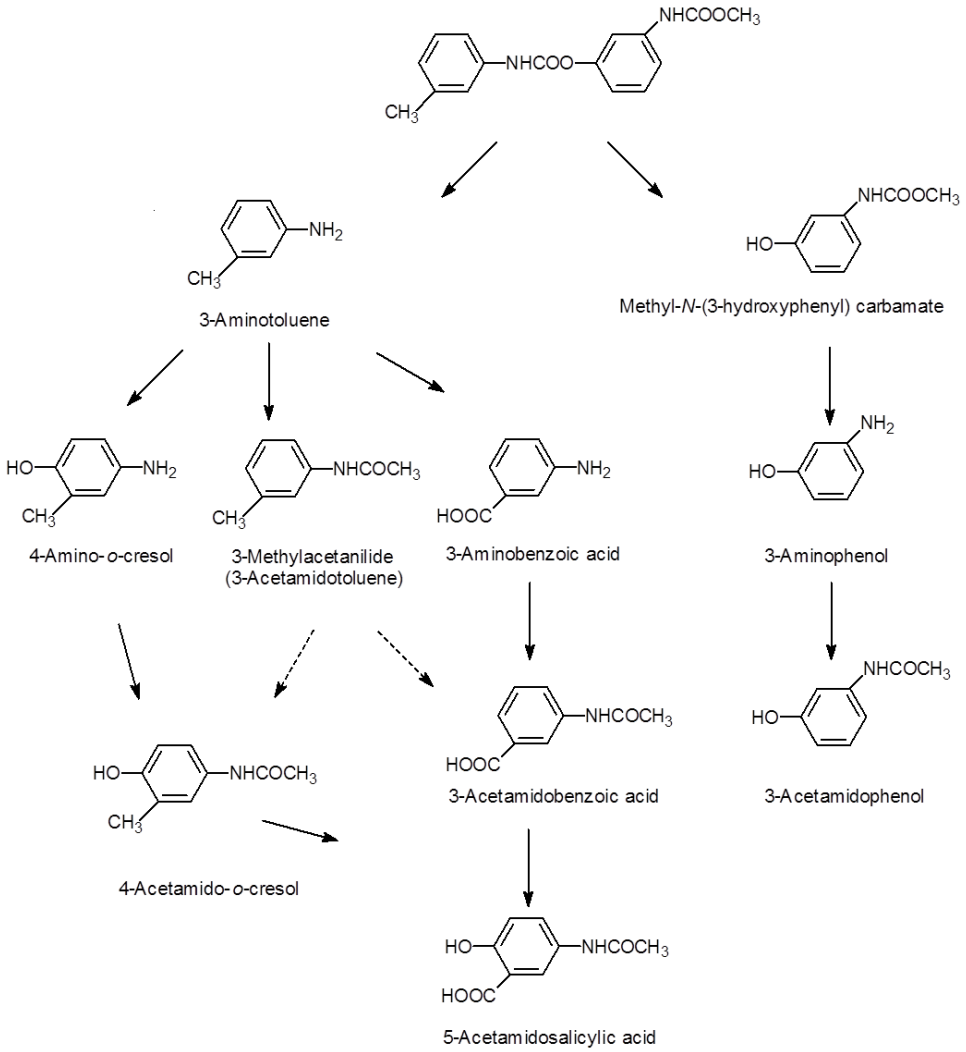


Figure 1 The proposed metabolic pathway of phenmedipham in rat

In vitro comparison of rat and human liver microsome metabolism of phenmedipham

The comparative metabolism of [Phenoxy-UL-^{14}C]Phenmedipham (^{14}C-Phenmedipham) was investigated in animal *in vitro* systems by incubating the test item with liver microsomes from male Wistar rats and humans with test durations of 0.5 and 1 hour (2015, RAR B.6.1.1/06). The identity of metabolites was not determined.

^{14}C-Phenmedipham was highly instable after incubation with buffer at 37°C and pH 7.4. A single radiolabelled compound (Pm-2) was produced and accounted for >53.2% of the radioactivity. Pm-2 was also detected in the liver microsome incubations from both species but in lower amounts, meaning that Pm-2 was subsequently metabolized by the liver microsome preparations.

In rat liver microsomes, 18.4% and 3.7% of the initial ^{14}C-Phenmedipham remained unchanged after 0.5 h and 1 h incubation, respectively. ^{14}C-Phenmedipham was metabolized towards a high number of metabolites. A total of 6 metabolites were detected in addition to the degradation product Pm-2, two of them (Pm-3 and Pm-5) were above 5% of the relative percentage (5.2-12.3% and 15.3-16.4%, respectively).

In human liver microsomes, a lower number of metabolites was found. The percentage of ^{14}C-Phenmedipham remaining after 0.5 h (47.0%) and 1 h (27.2%) incubation was remarkably higher as compared to the rat liver microsomes system indicating a slower metabolism rate of ^{14}C-Phenmedipham in human liver microsomes. From the four detectable metabolites formed by human liver microsomes (in

addition to the degradation product Pm-2), only one (Pm-3) was above 5% of the relative percentage (8.8-10.3%). This metabolite was also detected as one of the major metabolites in incubations with rat liver microsomes.

The slightly different metabolic pattern of ^{14}C-Phenmedipham when comparing rat and human liver microsomes relates, however, only to the lower number of metabolites detected in human liver microsomes. All metabolites, including the most abundant (Pm-3), were also detected in the tests with rat liver microsomes.

In summary, it can be assumed that in incubations with human liver microsomes no specifically different ^{14}C-Phenmedipham metabolites are formed in comparison with rat liver microsomes. No human-specific metabolites of ^{14}C-Phenmedipham were found in the liver microsomes system.

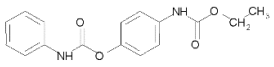
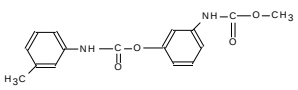
9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

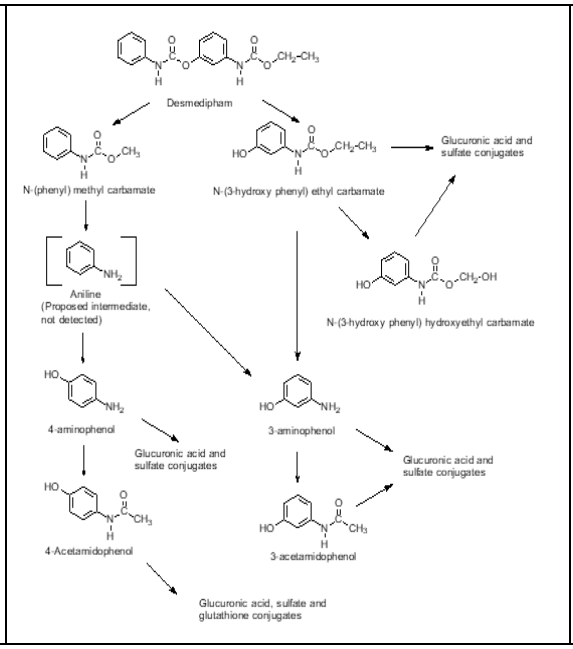
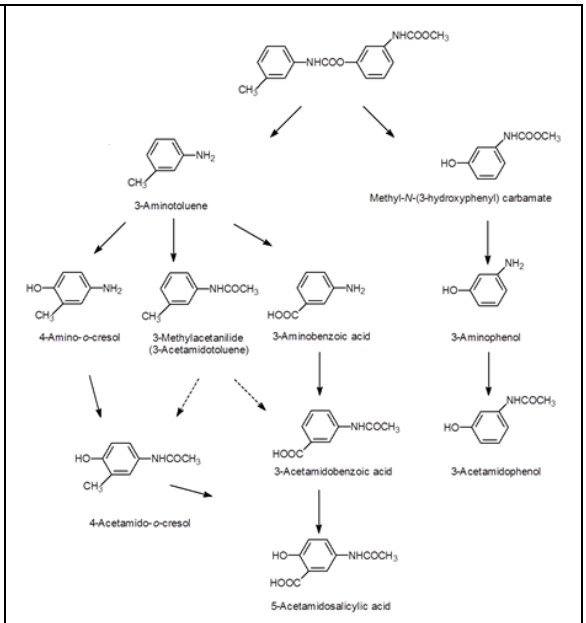
10 EVALUATION OF HEALTH HAZARDS

Read-across justification

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

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

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

Metabolic pathway		

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

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

Desmedipham and phenmedipham are metabolised to compounds which have aromatic amine structure. It is well known that aromatic amines have potential to induce formation of methemoglobin. Methemoglobin is a transformation product of normal oxyhemoglobin caused by the oxidation of Fe^{2+} to Fe^{3+}, thus converting ferroprotoporphyrin to the ferriprotoporphyrin form. MetHb binds oxygen more strongly than Hb and therefore does not effectively deliver oxygen to tissues. Based on available information on metabolites, 3-aminotoluene and aniline have potential to induce methemoglobin conversion and other hematotoxic effects (ECHA dissemination website). 3-aminotoluene (CAS 108-44-1, m-toluidine) has a harmonized classification as STOT RE 2 (effects on blood). Aniline (CAS 62-53-3) has a harmonized classification as STOT RE 1 (effects on blood), Carc cat 2 and Muta. cat 2. In addition, 3-aminophenol (CAS 591-27-5, p-aminophenol) and 4-aminophenol (CAS 123-30-8, m-aminophenol) have shown slight effects on blood (ECHA dissemination website; SCCS, 2011; SCCP, 2006).

The similarity of the effects seen in the toxicity studies seem to indicate that differences in the substances formed during the metabolism are not significant regarding their toxicity profiles. Desmedipham and phenmedipham in rats, mice and dogs show same type of effects on blood. The effects observed for both substances are consistent with effects pointing towards methemoglobinemia, leading to changes in red blood cell parameters and slight hemolytic anemia, increased activities of the bone marrow, kidney, liver and spleen - the organs mainly involved in the turnover of red blood cells - and compensatory hematopoiesis. For more detailed information see sections 13.10 (specific target organ toxicity -repeated exposure). The similarity in toxic effects is also supported by the similar level LOAEL-values of the higher tier studies (repeated dose toxicity and chronic toxicity). The available data do not indicate significant quantitative

differences in potency between substances. They seem to share a similar toxicity on blood and it is plausible they also share the same toxic mode of action.

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

Toxicology comparison of desmedipham and phenmedipham

Endpoint	Desmedipham	Phenmedipham
Acute oral	>2000 mg/kg (rat)	>2000 mg/kg (rat)
Acute inhalation	>7.4 mg/l (rat)	> 7 mg/l (rat)
Acute dermal	>2000 mg/kg (rat)	> 2500 mg/kg (rat)
Irritation – skin	No	No
Irritation - eye	No	No
Sensitisation	Slightly sensitising (GPMT)	No (study not acceptable)
Repeated dose toxicity (oral, rat and dog)	Rat (90 day oral, Wistar) LOAEL 5.2-5.6 mg/kg bw/day (methemoglobinemia) Rat (90 day oral, Wistar) LOAEL 24-27 mg/kg/bw day (↑ methemoglobinemia, ↑ hematopoiesis in the liver and spleen) Mice (28 day oral, NMRI) LOAEL 22-26 mg/kg/bw/dat (methemoglobinemia/haemolytic anemia, Heinz bodies ↑, hematopoiesis in spleen) Dog (90 day oral, Beagle) LOAEL 21.1. - 56.7 mg/kg bw/day (↑ Minimal/mild follicular epithelial hypertrophy in thyroids and increased thyroid weight) Dog (90 day oral, Beagle) LOAEL 53-57 mg/kg bw/day (marked iron deposits in the liver, ↑ erythropoiesis in the bone marrow)	Rat (90 day oral, Fischer 344) LOAEL 35 – 37 mg/kg bw/day (hemosiderin deposition (spleen), enlarged and/or black spleen, organ weight changes (spleen ↑, adrenals ↑), minimal anemia (reduced Hb, Hct, RBC; increased number of reticulocytes), changes in WBC (lymphocytes, neutrophils). Rat (90 day oral, Sprague-Dawley) LOAEL 30-33 mg/kg bw/day (mild decreases of red blood cell parameters (Hb, Hct, RBC), hemosiderin deposition (spleen, liver, kidneys), extramedullary hematopoiesis in the spleen, organ weight changes (spleen ↑ in males, uterus and thymus ↓ in females) Mice (8-week oral, Swiss CD-1) LOAEL: 623-699 mg/kg bw/day (methaemoglobinemia, increased liver weights (males), brown pigment in hepatic Kupffer cells, reduced Hb, Hct, RBC) Dog (60 day oral, Beagle) LOAEL 11-12 mg/kg bw/day (reduced BW gain in males, organ weight changes (thyroid ↑, testes ↓ ovaries ↑), possibly thyroid hypertrophy.
Genetic toxicity	Negative Ames, In vitro: OECD 476 +, OECD 473 (+/-), In vivo: OECD 474: negative, no germ cell tests	Negative Ames, Negative OECD 476, Positive OECD 473 (2), OECD 474 (+/-), negative OECD 483 (exposure not shown)
Carcinogenicity	Rat LOAEL 300 ppm (15.7 mg/kg bw/day) ↑ incidence of pituitary adenomas in Han Wistar males Mice LOAEL 30 ppm (5.8 mg/kg bw/day) ↑ incidence of ovarian tubular adenomas	Rat LOAEL 500 ppm (34 mg/kg bw/day) ↑ incidence of endometrial stromal sarcoma (Sprague-Dawley) ↑ incidence of pituitary adenomas in Han Wistar males (500 ppm/2500 ppm) Mice: no increase in tumour incidences
Reproductive	Sprague-Dawley, 2-gen: Maternal BW ↓ at	Sprague-Dawley, 2-gen: Maternal BW gain

toxicity	250 and 1250 ppm sperm (P) ↓ at 1250 ppm Developmental: ↑ motor activity, delayed eye opening and decreased sperm count at 250 and 1250 ppm, delayed onset of puberty at 1250 ppm.	and FC ↓ at 1000 ppm (86 mg/kg bw/day) Pups: none (minor decreases in BW/BW gain were observed) Reproduction: none
	Wistar, 2-gen: Parents: BW and food consumption ↓ at 1250 ppm. Hemolytic anemia, splenic weights ↑, erythropoiesis or hemosiderosis in spleen, erythroid hyperplasia in bone marrow, follicular hyperplasia in thyroid at 250, 1250 Reproduction: ↓ litter size at 250 and 1250 Pups: F1A BW ↓ of at 250, 1250 ppm	Wistar, 2-gen: Maternal BW/BW gain ↓ at 25 mg/kg bw/day Pups: Reduced BW (both sexes) at 1000 ppm (75 mg/kg bw/day) during lactation Reproduction: none
Developmental toxicity	Wistar, Maternal NOAEL >500 mg/kg Developmental: Increase in incidence of infarct of liver, bipartite ossification of sternebra at 100 and 500 mg/kg bw/day Increase in incidence incomplete ossification of interparietal bone and at 500 mg/kg bw/day (NOAEL 10 mg/kg)	Wistar: Maternal NOAEL <516 mg/kg): Reduced (corrected) BW gain and FC Pups (NOAEL <516 mg/kg): Reduced BW and incomplete ossification
	Wistar: Maternal NOAEL 10 mg/kg bw/day based on reduced body weight and corrected body weight gain, and reduced food consumption at 1000 mg/kg bw/day and slight ↓ bw gain at 100 mg/kg bw/day Developmental: ↑ number of fetuses with supernumerary rib at 100 mg/kg bw/day, (agnathia, microagnathia) observed at 100 and 1000 mg/kg bw/day	Wistar: Maternal NOAEL <150 mg/kg:based on reduced corrected BW gain Pups NOAEL <150 mg/kg): Runts were observed in all treated groups but not in controls
	Sprague Dawley: Maternal increase of spleen weight and discolored urine at 250 and 1000 mg/kg bw/day. Developmental: Increased incidence of subcutaneous haemorrhage at 250 and 1000 mg/kg bw/day. Fetal weight reductions, delayed ossifications at 1000 mg/kg bw/day (NOAEL (dev) 60 mgkg, NOAEL (mat) 60 mg/kg)	
	NZW rabbit: Maternal: ↓ body weight and food consumption at 270 (LOAEL), ↑ spleen weight at 90 and 270 (LOEL) Developmental: ↑ percentages of early embryonic death, fetal body weight ↓ at 270 (Developmental NOAEL: 30 mg/kg, maternal 30 mg/kg)	NZW rabbit: Maternal NOAEL 225 mg/kg bw/day: BW gain ↓ and FC ↓ at 1000 mg/kg bw/day Developmental NOAEL 225 mg/kg bw/day: Reduced BW and retarded ossification at 1000 mg/bw/day

Acute toxicity

10.1 Acute toxicity - oral route

10.2 Acute toxicity - dermal route

10.3 Acute toxicity - inhalation route

10.4 Skin corrosion/irritation

10.5 Serious eye damage/eye irritation

10.6 Respiratory sensitisation

10.7 Skin sensitisation

10.8 Germ cell mutagenicity

10.9 Carcinogenicity

Table 9: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results		Reference
		NOAEL	LOAEL Effects	
12-month oral toxicity study in rats OECD 452/453 (1981), GLP. Rat, Sprague-Dawley 20/sex/group Several tissues were not examined in histopathology (see description below).	Phenmedipham batch no. JS148, Purity: 98.5% 0, 60, 250, 1000 ppm M: 3, 15, 59 mg/kg bw/day F: 5, 19, 78 mg/kg bw/day Continuous in diet over 52 weeks	60 ppm M: 3 mg/kg bw/ day F: 5 mg/kg bw/ day	250 ppm Reductions in Hct, haemosiderin deposition (liver), blood pigment in urine Effects at 1000 ppm: Reductions in red blood cell parameters (Hb, RBC), haemosiderin deposition (kidneys and spleen), organ weight changes (spleen ↑, kidneys ↓), WBC and lymphocytes ↑, body weight gain reduction (females), follicular cysts in ovaries, lung alveolitis (males)	1988a B.6.5.1/01 M-146365-02-1 and 2000 M-199354-01-1 B.6.5.1./04

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**		**Reference**
		NOAEL	**LOAEL** **Effects**	
24-month oral carcinogenicity study in rats OECD 453, GLP Rat, Sprague-Dawley 50/sex/group (continuation of the above mentioned 12 month study)	Phenmedipham batch no. JS148 Purity: 98.5% 0, 60, 250, 1000 ppm M: 3, 13, 50 mg/kg bw/day F: 4, 17, 68 mg/kg bw/day Continuous in diet over 104 weeks	Systemic: 60 ppm M: 3 mg/kg bw/ day F: 4 mg/kg bw/ day Carcinogenic: >1000 ppm M: >50 mg/kg bw/ day F: >68 mg/kg bw/ day	250 ppm Focal pituitary hyperplasia (males), kidney focal simple transitional cell hyperplasia and pyelitis (males), urothelial mineral deposits and chronic progressive nephropathy (females), hemosiderin deposition (liver, spleen) Additional effects at 1000 ppm: Kidney focal papillary transitional cell hyperplasia (females), uterus stromal sclerosis, abnormalities in red cell morphology	1988b B.6.5.1/03 M-146366-02-1 (1st amendment to A62892) and histopathological extension (low and middle dose animals): 2000 B.6.5.1/04 M-199354-01-1
24-month oral carcinogenicity study in rats OECD 453, GLP Rat, Sprague-Dawley 50/sex/group Deviations: the study is compromised by the high incidence of cannibalised and autolysed animals plus high mortality and tumour rate in all groups.	Phenmedipham Purity: 97±1% 0, 60, 250,1000 ppm M: 3, 14, 55 mg/kg bw/ day F: 4, 18, 73 mg/kg bw/ day Continuous in diet over 104 weeks	Systemic: M: 60 ppm F: 250 ppm (supplementary NOAELs) M: 3 mg/kg bw/ day F: 18 mg/kg bw/ day Carcinogenic: >1000 ppm (supplementary NOAEL) M: >55 mg/kg bw/ day F: >73 mg/kg bw/ day	M: 250 ppm Reduced BW/BW gain, kidney pigmentation Additional effects at 1000 ppm: Kidney pelvic epithelial hyperplasia, cystitis of the urinary bladder, acute or subacute inflammation of the prostate, Kupffer cell pigmentation F: 1000 ppm Reduced BW/BW gain, endometrial sclerosis of the uterus, pulmonary alveolitis, Kupffer cell pigmentation, decreased hepatocyte vacuolation	1988c B.6.5.1/05 M-146387-03-1 (2nd amend-ment to A62913)
24-month oral toxicity/ carcinogenicity study in rats, GLP Rat, Sprague-Dawley,	Phenmedipham Lot No. U.S.A.-7038, Batch No. 260-121 Purity: assumed 100%	Systemic: 100 ppm (supplementary NOAEL) M: 5 mg/kg bw/	500 ppm Decreased body and organ weights (adrenals↓ and kidneys↓), changes in red blood cell parameters and increase in WBC	1980 B.6.5.1/06 M-145589-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**		**Reference**
		NOAEL	**LOAEL** **Effects**	
Charles River CD 60/sex/group	0, 20, 100, 500 ppm M: 1, 5, 28 mg/kg bw/ day F: 1, 7, 34 mg/kg bw/ day Continuous in diet over 104 weeks	day F: 7 mg/kg bw/ day Carcinogenic: 100 ppm F: 7 mg/kg bw/ day	Carcinogenicity, 500 ppm Increased incidence in endometrial stromal sarcoma	
24-month oral toxicity/ carcinogenicity study in rats, GLP, OECD 453 Rat, Han Wistar	0, 100, 500, 2500 ppm M: 4.6, 23.6, 117.6 mg/ kg bw/day F: 6.4, 33.1, 170.5 mg/ kg bw/day	Systemic: 100 ppm M: 4.6 mg/kg bw/day F: 6.4 mg/kg bw/day Carcinogenic: 500 ppm M: 23.6 mg/kg bw/day	500 ppm Slight anaemia and methaemoglobinaemia, histopathological changes in the spleen and kidneys secondary to increased erythrocyte turnover, organ weight change (spleen ↑); WBC, lymphocytes and platelets ↑ (males). Effects at 2500 ppm: Reduced BW gain and slightly reduced food consumption (females, MTD exceeded), organ weight changes (liver ↑, thymus ↑), histopathological changes (spleen: haemosiderosis and extramedullary hemopoiesis, liver: pigmented macrophages and Kupffer cells) Carcinogenicity, 2500 ppm Pituitary adenomas of pars distalis (males)	2004 B.6.5.1/07 M-240148-01-1
78-week dietary carcinogenicity study in CD-1 mice, GLP, OECD 451	0, 500, 2000, 7000 ppm M: 82, 331, 1167 mg/ kg bw/day F: 107, 443, 1532 mg/ kg bw/day	Systemic: M: 500 ppm F: 2000 ppm (supplementary for females) M: 82 mg/kg bw/day F: 443 mg/kg bw/ day	M: 2000 ppm Decreased incidence of hepatic periacinar vacuolation F: 7000 ppm Reduced BW gain (MTD exceeded), increased incidence of amyloidosis particularly in the adrenals, ovaries and spleen.	1991 B.6.5.1/08 M-146395-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results		Reference
		NOAEL	LOAEL Effects	
		(some histopathology not performed on remaining low and mid dose females) Carcinogenic: >7000 ppm	Effects at 7000 ppm in males: Reduced BW gain	
104 week dietary carcinogenicity study in CD-1 mice, GLP	0, 10, 100, 1000 ppm M: 1.1, 11, 110 mg/kg bw/day F: 1.2, 12, 117 mg/kg bw/day	100 ppm M: 11 mg/kg bw/ day F: 12 mg/kg bw/ day Carcinogenic: >1000 ppm	1000 ppm Organ weight increases (kidney and heart); also reduced MCHC and increased lymphocyte counts	1987 B.6.5.1/09 M-145666-01-1

10.10 Other studies relevant for carcinogenicity - Germ cell mutagenicity

Table 10: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Ames	98% phenmedipham		Negative. Tested strains Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100.	1987 B.6.4.1/02 M-145673-01-1
Ames	97% phenmedipham		Slightly positive in TA100 with and without S9. Tested strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100	1987 B.6.4.1/01 M-146383-01-1
Ames	97,7% phenmedipham		Negative: Salmonella typhimurium strains TA 1535, TA 1537, TA 98, TA 100, and TA 102.	2014 B.6.4.1/03 M-495889-01-1
In vitro chromosome aberration in human	99% phenmedipham	3 h treatment, 18 h harvest	Positive. With and without S-9 mix, Phenmedipham caused statistically significant increases in the proportion of aberrant cells. This response was seen in	1994 B.6.4.1/04 M-145797-01-

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
lymphocytes (OECD 473)			both the first and the second test, but only at toxic dose levels.	1
In vitro chromosome aberration in CHO (OECD 473)	97% phenmedipham	100 metaphases analysed	Positive. Dose-related **increases** in chromosomal aberrations were seen in CHO cells, in the absence and presence of S-9, at cytotoxic concentrations. However, among aberrations at high dose were also chromatid exchange which is not typical of cytotoxic aberrations (gap/break/fragment).	1986 B.6.4.1/05 M-146379-01-1
V79 Chinese hamster cell HGPRT Forward Mutation Assay	Puritynot reported, technical grade		Negative	1987 B.6.4.1/06 M-145664-01-1
Mouse lymphoma L5178Y assay (OECD 476)	97% phenmedipham	With and without S9	Negative	1986 B.6.4.1/07 M-146378-01-1
UDS in rat liver in vitro	phenmedipham technical, purity not reported		Did not induce significant increases in UDS. in rat primary hepatocytes were exposed for 18 h to phenmedipham at concentrations from 300 ng/ml to 2.5 ug/ml. However, treatments from 300 Mg/ml to 75 ng/ml were excessively toxic and treatments from 50 ng/ml to 2.5 ng/ml were prepared for UDS analysis.	1988 B.6.4.1/08 M-145675-01-1

Table 11: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Micronucleus test in mice by gavage	Purity not reported.	Doses: 100, 300 and l000 mg/kg, dosed twice separated by 24h. Sampling 6h after the second dose. 2000 PCE examined.	Negative. The ratio of polychromatic to normochromatic erythrocytes was comparable with the negative control value (approx. 1:1).	1978 B.6.4.1/07 M-146378-01-1
In vivo micronucleus test in mice (OECD 474), by gavage	phenmedipham, technical, purity not reported	15 g/kg dose tested for 24, 48 or 72 h.	Negative. PCE count was slightly reduced in the test groups and clearly in the positive control group. The low micronucleus count found in normochromatic erythrocytes can be taken to mean	1985 B.6.4.2/01 M-146384-01-1

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			that the micronuclei recorded in the polychromatic erythrocytes were not false micronuclei. The slightly reduced count of PCE (%) in the test groups shows that the dosage 15 g/kg, was on the borderline to affect erythropoiesis.	
EEC guideline for this test "Mammalian Germ Cell Cytogenetic Assay", (EEC-directive 79/831, B, 1985). This guideline is in agreement with the existing OECD guideline (GEN 85.5, fourth draft) by gavage. (mouse)	97% phenmedipham	50 metaphases /testis analysed (100/ animal), samples taken 12, 48 or 72h after dosing. Dose: 15 g/kg.	Negative. No depression of the spermatogenesis (reduction of mitotic relative to meiotic metaphases) was seen as a result of the dosing with phenmedipham. A clear depression of the spermatogenesis was seen in the mice dosed with cyclophosphamide. Frequencies of aberrant cells (gaps excl.) in the three test groups were not significantly different at 5% level when compared to the negative control group using Fishers Exact test.	1987 B.6.4.3/01 M-146385-01-1

10.10.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

Phenmedipham caused an increase in the number of chromosome aberrations *in vitro*. Phenmedipham did not lead to an increase in mutations in Ames assays. Following up in vivo negative results were obtained in two separate micronucleus assays in mice. In addition, phenmedipham did not cause chromosome aberrations when tested in mouse germ cells. Also mouse lymphoma assay resulted negative supporting the findings in Ames test that phenmedipham is not mutagenic *in vivo*.

Carcinogenicity studies in rats

104 week dietary carcinogenicity study in rats **(1988b B.6.5.1/03 M-146366-02-1)**, performed as an combined chronic toxicity\carcinogenicity study with 52 week findings reported under 1988a B.6.5.1/01 M-146365-02-1]**:** The carcinogenicity study in rat with oral route of administration was performed in compliance with OECD Guideline 453 and with the guideline for chronic toxicity in Directive 87/302/EEC, Part B, and according to the principles of GLP (OECD 1981).

Phenmedipham 104 week Dietary Study in Rats (project 43261, report M-146366-02-1). Groups of 50 male and 50 female Sprague-Dawley rats were dosed with 98.5% Phenmedipham via the diet at constant concentrations of 60 (low dose), 250 (intermediate dose) and 1000 (high dose) ppm, A control group of 50 males and 50 females received untreated diet. The corresponding doses in mg/kg were: M: 3, 13, 50 mg/kg bw/day F: 4, 17, 68 mg/kg bw/day.

Histopathological examination was carried out on all rats in the control and high dose groups and all premature decedents from the low and intermediate dose groups with the exception of seminal vesicles, vagina, spinal cord, thigh muscle, submandibular lymph node, eyes, tongue, aortic arch, duodenum, jejunum, caecum, rectum and nasal cavity). Male rat survival rates were 56% (control), 54% (low), 46% (intermediate) and 56% (high). The respective female rat survival rates were 42%, 54%, 54% and 56%.

In pituitaries, **focal hyperplasia** of the anterior lobe was statistically significantly increased in 250 ppm and 1000 ppm males. No increases were observed in pituitary adenomas [33/50 (0 ppm); 27/49 (1000 ppm)].

Urothelial hyperplasia (of the papilla and pelvis) was increased in number and severity in high dose females. Also the incidence of stromal sclerosis of the uterus was increased in the high dose females. Focal hyperplasia in the anterior lobe of the pituitary was increased in high dose males, but the incidence of anterior lobe adenomas was decreased in this same group.

A separately prepared extension to the original study (**2000 B.6.5.1/04M-199354-01-1)** was conducted in accordance with the OECD Principles of Good Laboratory Practice.A histological evaluation to the kidney, testis, epididymis and uterus of the remaining animals in the low and intermediate dose groups. All groups dosed with Phenmedipham showed a higher incidence of **interstitial cell adenoma** and **focal interstitial cell hyperplasia** when compared with the control group. Only the incidence of interstitial cell adenoma in the low dose group showed a statistically significant increase when compared with the control group ($P<0.05$).

Examination of background data from studies completed at the laboratory over 14 years (1978-1992) show that the control incidence for interstitial cell adenoma varied from 4.08% to 20% and that for focal interstitial cell hyperplasia varied from 0% to 18.75%. The incidences in this study for interstitial cell adenoma were 4.16%, 20.41%, 12.24% and 18.00% for control-high dose groups respectively. For focal interstitial cell hyperplasia the incidences were 6.25%, 14.28%, 10.20% and 16.00% for control-high dose groups respectively. It is noted that part of the historical data extends to more than 5 years from the conduct of the study (1998) and should thus used with caution. No further details on the HCD were available.

As for other non-neoplastic findings, the high dose female animals showed an increased incidence of stromal sclerosis of the uterus when compared with the control group. This was seen as a statistically significant increase at the mild grade only ($P<0.05$). Stromal sclerosis is not considered to represent either significant toxicity or to be a precursor to neoplastic changes, but rather a spontaneous phenomenon commonly seen in aging rats.

In the kidneys, the high dose female animals showed a statistically significant increase in the incidence of **focal papillary transitional cell hyperplasia**, when compared with the control group ($P<0.001$). The incidences of simple and nodular transitional cell hyperplasia were not increased in the female animals treated with phenmedipham. However, if all 3 types of focal transitional cell hyperplasia are merged together there is a statistically significant increase in the incidence of high dose females ($P<0.01$). There was a slight increase in the incidence of simple transitional cell hyperplasia in male animals from the intermediate dose group ($P<0.05$). However, the incidence of the papillary type was the same in control, low and high dose groups and the overall incidence when the 3 types are merged together is not statistically significant in the males.

Various forms of transitional cell hyperplasias occur as responses to bacterial infection, urinary tract toxins and carcinogens, calculi, or in association with renal papillary necrosis. Papillary hyperplasia, without atypia, is particularly associated with infective processes in the urinary tract but the female animals did not show evidence of an increase in incidence of pyelitis with increasing doses of phenmedipham in the present study or the original study. Tables 50 a-c list various histopathological findings from the (1988b B.6.5.1/03 M-146366-02-1).

Mortalities among the animals were fairly high. The high mortality rates extend over all groups, including controls, and seem not to be related to treatment. The whole study is characterised by a high incidence of tumors (mostly benign) in all groups of animals. The percentages of animals with tumors range from 81 to 96%. Because of the extremely high total tumor incidence, it is somewhat difficult to reliably assess possible treatment related effects. Comparison with historical control data on survival statistics of Sprague-Dawley

Crl:CD rats show that mortalities in this study are within the range observed in the test laboratory between 1980 and 1997 (survival percentages 23 – 70 % in males [mean: 48 ± 13 %], and 20 – 76 % [mean: 46 ± 13 %] in females). Incidences of benign and malignant tumors seen in all groups in this study are also comparable with historical background tumor incidence data for this strain of rats in the performing laboratory.

The original finding of a statistically non-significant increased incidence of interstitial cell adenomas in testes was upon re-examination found to be statistically significantly increased in high dose decedents, whereas the total incidence was higher in all treated groups compared to controls, however, only the findings in the low dose group reached statistical significance. The incidence of interstitial cell adenoma in premature decedents of 27% in the high dose group (1000 ppm, $p<0.05$) was outside the HCD range for 1978-1992 of 4-20%. No further details were available.

104 week dietary carcinogenicity study in rats **(1988c B.6.5.1/05 M-146387-03-1)**. Performed as an combined chronic toxicity\carcinogenicity study with 52 week findings reported under B.6.5.1/02 Everett et al. (1987).) Phenmedipham 104 Week Dietary Carcinogenicity Study in Rats (Project 431717). Groups of 50 male and 50 female rats were dosed for 104 weeks with Phenmedipham via the diet at dose levels of 0 (control), 60 (low dose), 250 (intermediate dose) or 1000 (high dose) ppm. The corresponding doses in mg/kg were: M: 3, 14, 55 mg/kg bw/ day; F: 4, 18, 73 mg/kg bw/ day. The study administration was performed in compliance with OECD Guideline 453. Histopathological examination was performed on all animals from the control and high dose groups and premature decedents on all organs and tissues fixed, with the exception of tongue, submandibular lymph node, nasal cavity, seminal vesicles and vagina. Histopathological examination of the kidneys, liver and lungs was performed on all other animals from low and intermediate dose groups. Survival rates of the male groups were 54% (control), 58% (low), 50% (intermediate) and 56% (high). The respective female survival rates were 62%, 66%, 58% and 64%.

There were a few neoplasms which were slightly increased in incidence in animals given 1000 ppm Phenmedipham. These were: **adrenal cortical tumours** [M: 0/49 (0), 0/21 (60), 0/24 (250), 2/49 (1000); F: 1/50 (0), 0/17 (60), 2/21 (250), 1/50 (1000)]**, adrenal malignant phaeochromocytomata** [M: 0/49 (0), 0/21 (60), 1/24 (250), 2/49 (1000); F: 2/50 (0), 0/17 (60), 0/21 (250), 0/50 (1000)]**, fibrosarcomata in the skin,** [M: 1/49 (0), 2/21 (60), 0/24 (250), 4/49 (1000); F: 1/50 (0), 0/17 (60), 0/21 (250), 0/50 (1000)] **malignant and poorly differentiated benign thyroid interstitial cell tumours** [M: 0/48 (0), 0/20 (60), 0/23 (250), 4/50 (1000); F: 0/47 (0), 0/17 (60), 0/21 (250), 0/50 (1000)] **and carcinomata of Zymbal's gland** (i.e. the auditory sebaceous gland) [M: 2/2 (1000); F: 1/1 (250)]. In all cases, however, the numbers involved were small (less than 5 of each type of tumour), the increase was confined to males, there was no corresponding increase in incidence of related benign and preneoplastic findings (where appropriate) and these general types of tumours occur spontaneously in aged rats. Three non-neoplastic histological findings showed a statistically significant increase in incidence in animals given 1000 ppm phenmedipham ($P<0.01$ and 0.05). These were (1) pelvic epithelial hyperplasia in the kidneys in males; (2) endometrial sclerosis in the uterus and (3) a small increase in alveolitis in females.

104 week chronic toxicity in Sprague-Dawley male and female rats. **(1980 B.6.5.1/06** M-145589-01-1**)**: 104-Week Chronic Toxicity in Male and Female Rats and Addendum (T27A). Sixty males and sixty female rats were dosed with 0, 20, 100 or 500 ppm of Phenmedipham (purity not reported). All preserved tissues from the sacrificed animals in the control and high dose groups were examined microscopically. Selected tissues preserved from the low- and mid-level groups were examined microscopically based upon compound-related findings noted in the high dose group. After 52 weeks, a group of 10 rats from each group was sacrificed and necropsied but only gross pathological findings were reported for these animals. All unusual lesions, palpable tissue masses and suspected tumors were examined from all animals. Survival rates for males were

as follows: 68% (control), 80% (low), 78% (mid) and 80% (high). Corresponding female survival rates were 78%, 75%, 72% and 63%. In the neoplastic findings, there were several singular tumours across all groups. The more frequent tumours, fibroadenoma or adenocarcinoma of the mammary gland, pituitary adenoma and cortical adenoma showed no relation to treatment. An increased incidence of **endometrial stromal sarcoma** was observed with summation of all incidences (terminal sacrifice and decedents) per group: 1/50 - 2% (0 ppm), 0/50 – 0% (20 ppm), 2/49 – 4.1% (100 ppm), 3/50 - 6% (500 ppm). The incidences, albeit few, show a dose-dependent increase and in the high dose group, the incidence of 6% is outside the historical control range given as 0-4.0% over an eight year period from 1983-1990; HCD was not collected before 1983 (the study was conducted 1977-1979) for endometrial stromal sarcoma (even though the table below gives a range of 1980-1990). The incidence in the mid dose group is just outside of the historical control range. However, as the historical control observations were collected 4-12 years after the study. Because the historical data extends to more than 5 years from the conduct of the study (**1980 B.6.5.1/06** M-145589-01-1) they should be used with caution. One of the incidences in the mid dose group (terminal kill) also had metastases to the mesentery, mesenteric lymph nodes and liver.

Phenmedipham Combined Carcinogenicity and Toxicity Study by Dietary Administration to Han Wistar Rats for 104 Weeks (**2004 M-240148-01-1 B.6.5.1/07)**. Three groups of 50 male and 50 female rats received 97% Phenmedipham, via the diet, at concentrations of 100, 500 or 2500 ppm. The control group received untreated diet. This comprised the carcinogenicity phase of the study. A further 20 male and 20 female rats were allocated to each group. These animals comprised the toxicity phase of the study and were sacrificed after the completion of 52 weeks of treatment. The achieved doses during the 104-week carcinogenicity phase were 4.6, 23.6 and 117.6 mg/kg bw/day for males and 6.4, 33.1 and 170.5 mg/kg bw/day for females receiving 100, 500 and 2500 ppm, respectively.

While the control male survival was 82%, the survival of the treated groups did not go below 80%. In the female control groups survival rate was 60% and 76% in low, 70% in mid and 62% in the high dose group.

An increased incidence in **adenoma of the distal part of the pituitary gland** in males receiving 2500 ppm was reported (incidences 7/50, (14%), at 0 ppm, 7/50 (14%) at 100 ppm, 12/50 (24%) at 500 ppm, and 19/50 (38%) at 2500 ppm respectively). When the tumour incidences were subjected to statistical analysis by time-to-tumour methods, the trend test was statistically significant when all groups were included in the analysis (p=0.002). When the results of the 2500 ppm dose group were removed, the trend test was no longer statistically significant (p=0.068). The pairwise comparison between the control and the 2500 ppm dose groups was statistically significant (p=0.013). The **adenoma incidence in the high dose males was 38%** Historical control adenoma incidences vary between 19-45% (average 31.6%). The histrorical control was collected from 17 studies conducted during 4/2001-6/2006; the number of animals studied varied from 49 to 60/study. Control females had an adenoma incidence of 46% and males 14%. There were no differences in the incidences of adenoma of the pars distalis in females. The lowest group incidence of adenoma of the pars distalis seen in control females in this study (46%) was also lower than the historical control range (female HCD range: 50-77%, average 64%).

In conclusion, histopathological examination after 104 weeks of treatment showed a statistically significant increase in the incidence of adenoma of the pars distalis of the pituitary in males at 2500 ppm. The relevance of the finding is, however, equivocal if historical control data are considered and may be discussed. No malign tumours were observed in any tissuesin this study The NOAEL for carcinogenicity is set to 500 ppm (equivalent to 24 mg/kg bw/day in males) based on an increased incidence of pituitary adenomas in males at 2500 ppm.

Carcinogenicity studies in Mice

78 Week dietary carcinogenicity study in mice. Groups of 50 male and 50 female CD-I mice were dosed for 78 weeks with 97% Phenmedipham via the diet at concentrations of 500 (low dose), 2000 (intermediate dose) and 7000 (high dose) ppm **(1991 B.6.5.1/08** M-146395-01-1**).** A further group of 50 males and 50 females received untreated diet to act as controls. Survival rates for males were 90% controls, 80% in all other groups. Female control group survival rate was 86% and 90% for low dose, 86% for the intermediate dose and 74% for the high dose group. In histology examination, intermediate and high male dose groups showed a statistically significantly reduced incidence of hepatic periacinar vacuolation. The high dose group females showed an increased incidence of amyloidosis particularly in the adrenals, ovaries and spleen. Statistically non-significant changes observed in males were: an increase in pulmonary adenoma at 500 ppm and 2000 ppm; an increase in malignant lymphoma at 500 ppm (4/50 compared to 1/50 in controls). A slight increase in histiocytic sarcomas was also noted in female mice treated with phenmedipham. The increases were not significant and the effect did not show a clear dose response. No preneoplastic findings were reported.
Overall, there was no evidence of carcinogenic potential in either sex. A NOAEL for carcinogenicity is >7000 ppm (corresponding to >1167 and >1532 mg/kg bw/day in males and females, respectively) as no neoplasms appeared to be related to the treatment with phenmedipham.

Technical Phenmedipham: Oncogenicity Study in the Mouse by Dietary Administration (**1987 B.6.5.1/09** M-145666-01-1**)**.

The study design consisted of four groups (including one untreated control group) each consisting of 52 males and 52 females as follows: control, 10, 100 or 1000 ppm of 99.3% phenmedipham was administered in diet to 52 males (102 weeks) and 52 females (104 weeks). The carcinogenicity study in mice with oral route of administration was performed in compliance with OECD Guideline 451 and according to the principles of GLP (OECD 1982).

Survival percentage in males ranged from 35% in controls to 27% in the high dose group, which can be considered fairly low. Female survival rate at termination varied from 71% in controls to 62% in the high dose group.

Neoplastic findings: Histopathological examination of the liver of all control and mice receiving 1000 ppm and of the macroscopically described abnormalities of the liver, showed increased incidences of **liver tumours** in high dose (1000 ppm) male mice when compared to controls (incidences 31% at 0, 51% at 1000 ppm, respectively Table 12. There was no significant difference in the incidence of malignant liver cell tumours between control and treated groups. The intergroup comparison between control and any treated groups of mice with benign and/or malignant liver cell tumours also did not show any significant difference. Moreover, the incidence of liver cell tumours seen in all groups in the study were within background control data.

The incidence of **lymphosarcomas** (including pleiomorphic lymphosarcoma) was slightly increased in phenmedipham treated high dose female mice, compared to controls. All the malignant lymphoma cases were multicentric. The differences in lymphosarcomas between controls and 10 ppm and 1000 ppm were not statistically significant (p=0.08 and 0.07, respectively). The incidences (21.1%) in multicentric lymphoreticular tumors were also within the range of historical background data in female controls (7.7%).

The incidences of liver cell tumors were reported as one, two or three tumors per liver. The number of benign liver tumors were more pronounced in high dose males, whereas the multiplicity of malignant liver tumors was similar in all groups of males. The table 12 below gives the total number of liver tumors in males

based on the finding of the re-examination of liver tumours. In females, the total number of liver tumors (benign and/or malignant) was similar between all groups.

Table 12 DISTRIBUTION OF LIVER TUMORS IN PREMATURELY DECEDENT AND TERMINALLY KILLED MALE MICE (total number of liver tumors in males based on the finding of the re-examination of liver tumours)

LESIONS	DOSE (ppm)			
	0	10	100	1000
Number of mice (livers) examined	51	52	52	51
Benign liver cell tumors	11	12	9	23
Malignant liver cell tumors	5	6	7	3
Liver cell tumors (total)	16	18	16	26

A NOAEL for carcinogenicity is >1000 ppm (corresponding to >110 and >117 mg/kg bw/day in males and females, respectively) as the incidence of neoplasms did not appear to be related to the treatment with phenmedipham.

The mortality rate in males was rather high in all groups (60-73%), with the highest death rate observed in the high dose group.

Summary of long-term toxicity and carcinogenicity studies

Eight long-term studies were included in the re-assessment of phenmedipham, one new study (**2004 M-240148-01-1 B.6.5.1/07**) and seven previously assessed studies (finalised from 1980 to 1991). Two of the studies were 52 week studies in rats which were combined to carcinogenicity studies, whereas the remaining six studies were carcinogenicity studies (four in rats and two in mice). All studies were performed as feeding studies.

The purity of technical phenmedipham was not stated in the study (**1980 B.6.5.1/06** M-145589-01-1) and so was assumed to be 100%. In the other studies, the purity ranged from 97±1% to 99.3%.

Mainly hematological changes (decreased Hb, HCT and RBC), increased total white cell counts and lymphocyte counts) were noted in the 52 week rat study (1988a B.6.5.1/01 M-146365-02-1), mostly at the highest dose of 1000 ppm (59-84 mg/kg bw/day), but partly also at 250 ppm (15-20 mg/kg bw/day). The toxicity phase of the study by **2004 M-240148-01-1 B.6.5.1/07** generally confirmed the findings by the above 52 week study in terms of reduction in red blod cell parameters, increased lymphocyte and white blood cell counts, hemosiderin deposition (liver, spleen), splenic hematopoiesis and increased spleen weights at the highest dose (137-196 mg/kg bw/day). **2004 M-240148-01-1 B.6.5.1/07** also found congestion in the spleen of males from 6 mg/kg bw/day and increased relative liver weights and reduced kidney weight (absolute and relative) at the highest dose.

Two of the 2-year rat studies (1988b B.6.5.1/03 M-146366-02-1 and 1988c B.6.5.1/05 M-146387-03-1) had very high mortality rates in all groups of test animals, treated or controls. In the second 2-year study (1988c B.6.5.1/05 M-146387-03-1), unacceptably high incidences of autolysed and cannibalised animals were observed (in excess of 10%). High incidences of all types of tumors, both benign and malignant, were observed in all groups of both 2-year studies. The two 2-year studies in question were performed in the same laboratory practically at the same time, using the same strain of animals from the same supplier, which may explain the similarities in the overall performances of the tests. The percentages of survival in the 1988b

B.6.5.1/03 M-146366-02-1 rat study were 46 – 56% in males and 42 – 56% in females. The corresponding figures in 1988c B.6.5.1/05 M-146387-03-1 were 50 – 58% in males and 58 – 66% in females. The lowest survival rates in males (46%) and females (42%) are well within the historical control values (1980-1997: 48 ± 13% in males and 46 ± 13% in females) from the same test laboratory. **Increased stromal/endometrial sclerosis** of the uterus in high dose females was observed in both of the almost identical 2-year studies (1988b B.6.5.1/03 M-146366-02-1) and (1988c B.6.5.1/05 M-146387-03-1). From 13-17 mg/kg bw/day, **focal pituitary hyperplasia** (males) was observed as well as various kidney effects (focal simple transitional cell hyperplasia and pyelitis in males, chronic progressive nephropathy and urothelial mineral deposits in females); **focal papillary transitional cell hyperplasia** was increased in high dose females (1988b, 2000). In the other study, **pelvic epithelial hyperplasia** was seen in high dose males (1988c), whereas pulmonary alveolitis and decreased hepatocyte vacuolation was observed in high dose females (73 mg/kg bw/day). Pelvic epithelial hyperplasia was also increased in males at 118 mg/kg bw/day in the carcinogenicity phase of the study by (2004 **M-240148-01-1 B.6.5.1/07**) with increases also in pelvic epithelial mineralisation, whereas the incidence of kidney interstitial inflammatory cells was observed in males from 24 mg/kg bw/day.

Hematological changes and pigment deposits in liver, spleen and kidneys were again noted in the carcinogenicity phase of the two rat studies (1988b B.6.5.1/03 M-146366-02-1 and 1988c B.6.5.1/05 M-146387-03-1); in case of 1988c slightly increased hematopoiesis in sternum was observed in treated females. In the 2-year study by (2004 **M-240148-01-1 B.6.5.1/07**), methemoglobinemia was found to be reversible at the lower doses, however, methemoglobin was still increased (up to 123%, 0.87% MetHb) after 78 and 104 weeks at 118-171 mg/kg bw/day. Furthermore, the carcinogenicity phase of this study showed reductions in red blod cell parameters, increases in reticulocytes and increased anisocytosis and hyperchromasia. Also, lymphocytes, white blood cells and platelets were increased. Spleen weight increases were observed from 24 mg/kg bw/day, and liver and thymus weights were increased at 118-171 mg/kg bw/day. Hemosiderin deposition was seen in liver, spleen and kidneys at 118-171 mg/kg bw/day, as well as extramedullary haemopoiesis and congestion in the spleen. The systemic NOAEL for the study (2004 **M-240148-01-1 B.6.5.1/07**) is 5-7 mg/kg bw/day. The supplementary 2-year rat study (**1980 B.6.5.1/06** M-145589-01-1) showed decreases in body weights and relative adrenal and absolute kidney weights at 28-34 mg/kg bw/day. No significant changes between controls and treated groups were observed in gross pathology or histopathological examinations. Transient changes in several hematological parameters were observed during the study. The supplementary systemic NOAELs in the study by (**1980 B.6.5.1/06** M-145589-01-1) were 5 mg/kg bw/day in males and 7 mg/kg bw/day in females.

Increased incidences of neoplasms in phenmedipham treated rats were observed in two studies. The incidence of **endometrial stromal sarcoma** was increased at 34 mg/kg bw/day (6% vs. 2% in controls, historical control range of the performing laboratory 0-4%) in the study by **1980 B.6.5.1/06** M-145589-01-1. In the study (2004 **M-240148-01-1 B.6.5.1/07**), the incidence of **adenomas in the pars distalis of the pituitary** in male Wistar rats showed a dose-dependent increase that was found to be statistically significant (time-to-tumour method) at 118 mg/kg bw/day (38% vs. 14% in controls, all animals). The increase was most pronounced in prematurely decedent males (86% vs. 11% in controls) and the adenomas were reported to be the cause of death. The remaining long-term studies in rats were performed using Sprague-Dawley rats that have a spontaneous incidence of pituitary adenomas (generally >50%) and no effect of treatment on pituitary neoplasms was observed. (Pituitary effects may be endocrine-mediated, however, phenmedipham was found in the brain after single exposures in the ADME studies).

Markedly reduced body weight gains and reduced absolute kidney weights were noted in the 78 week mouse study (1991 B.6.5.1/08 M-146395-01-1) at 7000 ppm (1167 – 1532 mg/kg bw/day). Amyloidosis of several organs in females was also noted at this dose; all animals at the lower doses were, however, not investigated for these findings. In males, decreased hepatic periacinar vacuolization was seen from 331 mg/kg bw/day. No proper hematological analyses were performed in this study, therefore possible hematological effects caused by phenmedipham was not fully evaluated. The systemic NOAEL for males is 82 mg/kg bw/day and a temporary systemic NOAEL for females is 443 mg/kg bw/day. In the 2-year mouse study by (**1987 B.6.5.1/09** M-145666-01-1), the number of premature mortalities in males was rather high (60-73%). Some hematological changes (decreased MCHC, increased lymphocyte counts) were observed in the 2-year oncogenicity study in mice at 100 ppm (11 – 12 mg/kg bw/day) and 1000 ppm (110 – 117 mg/kg bw/day). In this study doses up to 110 – 117 mg/kg bw/day did not cause increases in methemoglobin levels in mice (in week 103). Some organ weight changes were noted at 1000 ppm (increased relative weight of kidney and heart). Histopathological examination showed an increase in benign liver tumors and lymphosarcomas (multicentric tumors), but the differences were not statistically significant. The systemic NOAELs, based on organ weight changes, were 11 mg/kg bw/day for males and 12 mg/kg bw/day for females.

No treatment-related neoplasms were observed in the two carcinogenicity studies in mice.

Target organs in the long-term toxicity/carcinogenicity studies were: blood system, kidney, liver, spleen, uterus, ovaries, pituitary, adrenals lungs and prostate. The overall systemic NOAEL for long-term toxicity is 3 mg/kg bw/day, based on the 2-year study by 1988b B.6.5.1/03 M-146366-02-1 with extension analysis by (2000). This value is supported by five other studies including the most recent carcinogenicity study from 2004 with NOAELs in the range of 3-5 mg/kg bw/day (1988c B.6.5.1/05 M-146387-03-1, **1980 B.6.5.1/06** M-145589-01-1, 2004 **M-240148-01-1 B.6.5.1/07**). The overall NOAEL for carcinogenicity is 7 mg/kg bw/day, based on the study by **1980 B.6.5.1/06** M-145589-01-. Table 52 and 53 below summarise selected tumours found in rats and mice carcinogenicity studies. Table 54 complies the tumours considered in the classification

Table 13: Selected incidences of neoplastic microscopic leasons in rats (including interim and terminal sacrifices and decedents)

Sex	**Males**				**Females**			
Feeding dose, ppm	**0**	**20**	**100**	**500**	**0**	**20**	**100**	**500**
Endometrial stromal sarcoma					1/50	0/50	2/49	3/50
Endometrial polyp/fibrovascular/uterine polyp					3/50 (6%)	1/50 (2%)	2/49 (4%)	5/50 (10%)
Feeding dose	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Adenomas of pars distalis	7/50 (14%)	7/50 (14%)	12/50 (24%)	19/50 (38%)*	23/50 (46%)	33/50 (66%)	26/50 (52%)	26/49 (53%)
Focal hyperplasia	6/50 (12%)	8/50 (16%)	3/50 (6%)	8/50 (16%)	3/50 (6%)	0/50 (0%)	1/50 (2%)	2/50 (4%)

*Statistically significant: p=0.013

Incidence of pituitary adenoma of pars distals in male historical control groups: 12-36.7%. Historical incidence of malignant liver tumours: 9-20/52 mice examined. Lymphosarcoma: 5-18/52.

Table 14: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Sprague-Dawley	Pituitary adenoma	No	No	No	single, in males only	No	Oral (relevant to human)	Possibly hormonal, relevant to humans
Wistar	Endometrial stromal sarcoma	No	Yes	No	-	No	Oral (relevant to human)	Possibly hormonal, relevant to humans

Carcinogenicity in experimental animals

Incidence of pituitary adenomas of the pars distalis was significantly increased in Han Wistar male rats at high dose (2500 ppm, 2004 **M-240148-01-1 B.6.5.1/07**). When the tumour incidences were analysed by time-to-tumour methods, the trend test was statistically significant when all groups were included (p=0.002). When the 2500 ppm dose group was excluded, the trend test was no longer statistically significant (p=0.068), Pair-wise comparison between the control and the 2500 ppm dose groups was statistically significant (p=0.013). There were no differences in the incidences of adenoma of the pars distalis in females. Concerning preneoplastic changes, focal hyperplasia in the anterior lobe of the pituitary was increased in high dose Sprague-Dawley males (1988b B.6.5.1/03 M-146366-02-1), but the incidence of anterior lobe adenomas was decreased in this same group and since hyperplasia in the pituitary is considered to be pre-neoplastic, these findings were considered together. This showed that there is little difference in the incidence of adenoma/hyperplasia between control, mid and high dose males. Based on Guidance on the Application of the CLP Criteria Version 5.0 – July 2017 (ECHA) Sprague-Dawley rats have a high spontaneous incidence of pituitary adenomas for which hyperplasia could be seen as first indication.

Desmedipham is a structurally, physico-chemically and toxicologically similar substance to phenmedipham (See section 10, read-across justification). Incidences of pituitary adenomas of pars distalis were also slightly increased at mid and high dose in Han Wistar male rats after two years desmedipham treatment. There were some indications that the pituitary tumors appeared earlier also in desmedipham treated males compared to controls (CLH report for desmedipham, Finland). The endometrial stromal sarcoma (ESS) incidence increased (**1980 B.6.5.1/06** M-145589-01-1) in Sprague-Dawley rats. In the carcinogenicity phase of the study, an ESS had the following incidences: 1/50 - 2% (0 ppm), 0/50 – 0% (20 ppm), 2/49 – 4.1% (100 ppm), 3/50 - 6% (500 ppm). The high dose group incidence of 6% is outside the historical control range given as 0-4.0% over an eight year period from 1983-1990; HCD was not collected before 1983 for endometrial stromal sarcoma although the a range of 1980-1990 is cited. It is noted that part of the historical data extends to more than 5 years from the conduct of the study (1980) and should thus used with caution. The incidence in the mid dose group is outside of the historical control range by one tenth of a precentage. Although the highest dose was outside the HC incidence range, there does not seem to be a clear positive trend over the dose range. No increase in the stromal sarcomas were found in desmedipham treated animals.

Neither of the tumour types were increased in the mouse studies.

Mechanism of action

While there were positive results in two OECD 473 (chromosome aberration *in vitro*) there is no evidence of genotoxicity *in vivo*. These results were not confirmed two in vivo (OECD 474 (Mammalian Erythrocyte Micronucleus Test *in vivo*). However, in one study did not provide sufficient evidence that the bone marrow had been exposed (change in polychromatic (PCE) to normochromatic (NCE) erythrocyte ratio was not demonstrated), whereas when dosing 15000 mg/kg there was a reduction of PCE indicating exposure. Similarly, one might question whether the test substance reached the testes in the germ cell assay. However, the distribution study performed with rats showed that both the bone and testes were exposed after oral doses of 20 mg/kg or 1000 mg/kg. Also, in the studies B.6.4.1/07 M-146378-01-1 (1978) and B.6.4.2/01 M-146384-01-1 (1985) a considerably larger dose was given (15000 mg/kg), which would indicate that the negative results of these studies are not false negatives and that phenmedipham is not genotoxic *in vivo*.

Pituitary adenomas and endometrial sarcomas are typically hormonally related tumours. Phenmedipham has shown effects on some hormone sensitive organs (e.g. decreases in uterus, prostate and thymus weights and increases in adrenals, testes and ovaries weights in rats (See RAR B.6.8.3. Sudies on endocrine disruption). Therefore, although no definite conclusion can be drawn it is plausible that the occurrence of these tumours is hormonally related, namely by disturbed homeostasis of the hypothalamus-pituitary-gonad (thyroid) axis. This mechanism is relevant to humans.

Toxicokinetics

Phenmedipham is efficiently absorbed orally and widely distributed but efficiently excreted excreted as 3-acetaminophenol and various glucuronide acetaminophenol conjugates. No significant differences in toxicokinetic characteristics are expected to occur between rats and humans. See section 9 for details on toxicokinetics.

10.10.2 Comparison with the CLP criteria

Consideration of category 1A

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

Consideration of category 1B

Concerning the animal studies, the available information indicates there might be evidence that "*a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in …(b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.*" However, incidence of the malignant tumours (stromal sarcoma of the uterus) at the highest dose was only marginally over the historical control range of the performing laboratory. Moreover, the incidence was only slightly higher compared to the control group (1/50 vs. 3/50). Thus it cannot be concluded that the"*malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.* Therefore, the available information from test animals cannot be considered sufficient to classify phenmedipham as carcinogenicity category 1B.

Consideration of category 2

For concluding that a substance is compatible with a classification as a category 2 carcinogen, the test animal studies must show there is at least **limited** evidence of carcinogenicity, i.e., "*the data suggest a carcinogenic effect but are limited for making a definitive evaluation…* The findings could warrant classification for

category 2 on the basis of Category 2 criteria: *(c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential;*

We conclude that there is limited evidence of carcinogenicity for phenmedipham based on the increased incidences of the slightly increased incidences of pituitary adenomas and endometrial stromal sarcomas in rat. Classification in carcinogenicity category 2 is proposed.

10.10.3 Conclusion on classification and labelling for carcinogenicity

In conclusion, the available information can be considered sufficient evidence to classify the substance as:

Carcinogenicity 2, H351: Suspected of causing cancer.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Six carcinogenicity studies with phenmedipham were available, four in rats and two in mice. The DS proposed classification in Category 2 based on increased incidence of pituitary adenomas (male rats, study 6.5.1/07) and endometrial stromal carcinomas (rats, study 6.5.1/06).

Comments received during public consultation

Four MSCAs and 1 Industry association provided their comments.

Two MSCAs clearly supported classification in Category 2 while the other 2 MSCAs indicated this to be a borderline case between Category 2 and no classification.

Industry argued against classification. Regarding the uterine tumours, they pointed out lack of statistical significance in a trend test and a relatively high background incidence according to published historical control data (HCD). As for the pituitary adenomas, industry emphasised the high variability demonstrated by a relevant HCD, no increase in precursor lesions or adenocarcinomas and lack of a tumour increase in females. Further, they challenged the DS' assumption that the mode of action (MoA) of both tumours is related to disturbed homeostasis of the hypothalamus-pituitary-gonad (thyroid) axis.

In their responses to the comments, the DS agreed that the case is borderline and that a hormonally mediated MoA has not been clearly demonstrated.

Assessment and comparison with the classification criteria

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

Carcinogenicity studies		
Type of study; Reference; Year	**Method**	**Observations**
Rat		
2-year chronic toxicity/carcinogenicity, dietary B.6.5.1/07 2004	OECD TG 453 GLP Strain: Han Wistar Doses: 0, 100, 500, 2 500 ppm; equivalent to 4.6/6.4, 24/33, 118/171 mg/kg bw/d (m/f) 1-year: 20/sex/group 2-year: 50/sex/group	<u>Non-neoplastic findings</u> 2 500 ppm (118/171 mg/kg bw/d): • ↓ bw gain (f by 26 %, stat. sign.; males, by 7 %, not stat. sign.); ↓ food consumption (females, by 8 %) • ↓ Hb (by up to 10/12 % m/f); ↑ MetHb (up to 2-fold, max. 0.9 %), no Heinz bodies; ↑ reticulocytes; ↑ anisocytosis and hyperchromasia; ↑ lymphocytes • ↑ spleen weight (males, by ca. 20 %) • ↑ incidence of pigment in Kupffer cells, haemosiderosis in the spleen, extramedullary haematopoiesis in the spleen, splenic congestion, renal tubular pigmentation, renal pelvic epithelial mineralization and hyperplasia (males), renal interstitial inflammatory cells (males) 500 ppm (24/33 mg/kg bw/d): • ↑ incidence of renal interstitial inflammatory cells (males) 100 ppm (4.6/6.4 mg/kg bw/d): no adverse effects <u>Neoplastic findings</u> 2 500 ppm: • **Pituitary adenoma** (males) ≤ 500 ppm: no neoplastic effects
2-year chronic toxicity/carcinogenicity, dietary B.6.5.1/01,03,04 1988	OECD TG 453 GLP Strain: Sprague-Dawley Doses: 0, 60, 250, 1 000 ppm; equivalent to 3.1/4.1, 13/17, 50/68 mg/kg bw/d (m/f) 1-year: 20/sex/group 2-year: 50/sex/group	<u>Non-neoplastic findings</u> 1 000 ppm (50/68 mg/kg bw/d): • ↓ Hb (by ca. 7 % after 1 year) • ↑ incidence of haemosiderin deposition in Kupffer cells and renal tubular cells, urothelial hyperplasia (females), focal pituitary hyperplasia (males) (but no increase when hyperplasia and adenomas are combined), uterine endometrial stromal sclerosis <u>Neoplastic findings</u> None
2-year chronic toxicity/carcinogenicity, dietary B.6.5.1/02,05 1988 Conducted by the same	OECD TG 453 GLP Strain: Sprague-Dawley Doses: 0, 60, 250, 1 000 ppm; equivalent to 3.3/4.3, 14/18,	<u>Non-neoplastic findings</u> 1 000 ppm (55/73 mg/kg bw/d): • ↓ bw (females, by 10 % after 1 year) • ↓ Hb (by ca. 7 % after 1 year) • ↑ incidence of haemosiderin deposition in Kupffer cells (m/f) and renal tubular cells (males), renal

laboratory as B.6.5.1/03 with animals of the same strain and source; the purity of the test substance was different	55/73 mg/kg bw/d (m/f) 1-year: 20/sex/group 2-year: 50/sex/group Deficiency: high incidence of autolysed or cannibalised animals (ca. 20 % of the males across groups in the 2-year study)	pelvic epithelial hyperplasia (males), uterine endometrial sclerosis Neoplastic findings None (several tumour types discussed by the DS)	
2-year chronic toxicity/carcinogenicity, dietary B.6.5.1/06 1980	OECD TG 453 GLP Strain: Sprague-Dawley Doses: 0, 20, 100, 500 ppm; equivalent to 1.1/1.4, 5.5/6.8, 28/34 mg/kg bw/d (m/f) 1-year: 10/sex/group 2-year: 50/sex/group	Non-neoplastic findings 500 ppm (28/34 mg/kg bw/d): • ↓ bw (females, by 6 % at termination) • ↓ Hb (females) Neoplastic findings None (endometrial stromal sarcoma discussed by the DS)	
Mouse			
18-month carcinogenicity, dietary B.6.5.1/08 1991	OECD TG 451 GLP Strain: CD-1 Doses: 0, 500, 2 000, 7 000 ppm; equivalent to 82/107, 331/443, 1 170/1 530 mg/kg bw/d (m/f) 50/sex/group	Non-neoplastic findings 7 000 ppm (1 170/1 530 mg/kg bw/d): • ↓ bw (females, by 10 % at termination) • ↑ incidence of amyloidosis (females) Neoplastic findings None	
2-year carcinogenicity, dietary B.6.5.1/09 1987	OECD TG 451 GLP Strain: CD-1 Doses: 0, 10, 100, 1 000 ppm; equivalent to 1.1/1.2, 11/12, 110/117 mg/kg bw/d (m/f) 52/sex/group	Non-neoplastic findings No adverse effects Neoplastic findings None	

Rat carcinogenicity study B.6.5.1/07

The top dose selection in this study (2500 ppm) was based on a dose range-finding 90-day study (B.6.3.2/06), where a body weight gain reduction of ca. 15 % was observed at 3 000 ppm. A dose of 10 000 ppm in the 90-day study caused a reduction in body weight

and food consumption by ca. 20 % and Hb reduction by up to 18 %. Although the maximum tolerated dose (MTD) does not seem to have been reached in the carcinogenicity study itself, some toxicity was present at the top dose (haematotoxicity, haemosiderosis, histopathological findings in the kidney, minor effects on body weight in females) and taking into account the findings of the dose range-finding study, the top dose selection is considered acceptable.

The only neoplastic finding was increased incidence of pituitary adenoma in top dose males. The incidences are provided in the table below. The increase was statistically significant. The top dose incidence remained within a relevant HCD range and was close to the HCD mean. However, the concurrent control incidence was below the HCD range and the incidence at the low dose shows that the concurrent control was not aberrant. This, together with the apparent dose-response relationship, indicates that the increase was treatment-related. There was no increase in hyperplasia in males and no histopathological changes in the pituitary of females.

Neoplastic and hyperplastic findings in the pars distalis in study B.6.5.1/07					
Dose (ppm)	**0**	**100**	**500**	**2 500**	**HCD[a]**
Dose (mg/kg bw/d) (m/f)	0	4.6/6.4	24/33	118/171	
Males					
No. of animals examined	50	50	50	50	
Adenoma	7 (14 %)	7 (14 %)	12 (24 %)	19* (38 %)	Mean: 32 % Range: 19-45 %
Adenocarcinoma	0	0	0	0	
Focal hyperplasia	6	8	3	8	
Females					
No. of animals examined	50	50	50	49	
Adenoma	23	33	26	26	
Adenocarcinoma	3	0	1	2	
Focal hyperplasia	14	10	10	5*	

* Statistically significant difference from control, p≤0.05

[a] 17 studies within 5 years of the current study (studies starting 2001-2006; the current study started in 2001), the same laboratory, strain and supplier

Pituitary tumours were more frequent among top dose male early decedents than among control decedents, and pituitary tumours were a factor contributing to death of these animals according to the pathology report. There was no obvious group difference in females in this regard.

Tumours of pituitary pars distalis in study B.6.5.1/07: time of the finding, contribution to unscheduled deaths				
Dose (ppm)	**0**	**100**	**500**	**2 500**
Males				
Adenoma in animals sacrificed after 52 weeks	0/20	0/20	1/20 (1 +)	1/20 (1 +)
Adenoma in animals sacrificed or dying between week 52 and 104	1/9 (1 +++)	3/8 (3 +++)	5/10 (5 +++)	6/7 (6 +++)
No. of animals for which pituitary adenoma was listed as a factor contributing to unscheduled death	1 (1 s)	3 (3 s)	5 (4 s)	6 (5 s)
Adenoma in animals sacrificed after 104 weeks	6/41 (2 +, 2 ++, 2 +++)	4/42 (2 +, 1 ++, 1 +++)	7/40 (2 +, 4 ++, 1 +++)	13/43 (4 +, 6 ++, 3 +++)
Week of death of animals sacrificed or dying between week 52 and 104	81	72, 88, 102	73, 78, 87, 94, 97	83, 93, 97, 98, 100, 102
Females				
Adenoma in animals sacrificed after 52 weeks	0/20	1/20	1/19	1/19
Adenoma or adenocarcinoma in animals sacrificed or dying between week 52 and 104	12/20 (9 a, 3 c)	9/12 (9 a)	9/15 (8 a, 1 c)	11/19 (9 a, 2 c)
No. of animals for which pituitary tumour was listed as a factor contributing to unscheduled death	7 (6 s)	6 (5 s)	8 (6 s)	7 (7 s)
Adenoma in animals sacrificed after 104 weeks	14/30	24/38	18/35	17/31

Size of tumour: +, not apparent on macroscopic investigation; ++, mass apparent macroscopically, no compression of the brain; +++ mass compressing the brain

s = pituitary adenoma was the sole factor contributing to death listed in the pathology report for the animal

a = adenoma, c = carcinoma

Rat carcinogenicity studies B.6.5.1/03, B.6.5.1/05 and B.6.5.1/06

These three carcinogenicity studies used top doses of 1 000 ppm, 1 000 ppm and 500 ppm respectively. The general toxicity at the top doses was rather limited and as the MTD in 90-day studies seems to lie around 5 000 ppm (B.6.3.2/04, /05, /06), none of these three carcinogenicity studies is considered to have fully investigated the carcinogenicity potential of phenmedipham.

While no treatment-related neoplastic findings were observed in study B.6.5.1/03, the DS pointed out some small increases in incidences of several tumours in study B.6.5.1/05,

conducted by the same laboratory with animals of the same strain and source as B.6.5.1/03, but presumably with a test substance of a different batch or source. The neoplastic findings from study B.6.5.1/05 are presented in the table below. As none of the increases was statistically significant on pairwise comparison, the increases are limited to one sex and there was no increase in incidence of these tumours in other rat carcinogenicity studies at comparable or higher doses (B.6.5.1/03, /07), RAC does not consider these findings sufficient for classification.

Neoplastic findings in study B.6.5.1/05 (only premature decedents examined histopatologically in the low and mid-dose group)

	Males				**Females**			
Dose (ppm)	**0**	**60**	**250**	**1 000**	**0**	**60**	**250**	**1 000**
No. of animals examined	48-49	20-21	23-24	49-50	47-50	17	21	50
Adrenal cortical tumour	0	0	0	2	1	0	2	1
Adrenal malignant phaeochromocytoma	0	0	1	2	2	0	0	0
Fibrosarcoma of the skin	1	2	0	4	1	0	0	0
Thyroid interstitial cell carcinoma	0	0	0	2	0	0	0	0
Thyroid interstitial cell adenoma, poorly differentiated	0	0	0	2	0	0	0	0
Thyroid interstitial cell adenoma, well differentiated	4	0	0	2	5	1	2	2

The increased incidence of endometrial stromal sarcoma in study B.6.5.1/06 (incidences 1, 0, 2 and 3 out of 49-50 animals at 0, 20, 100 and 500 ppm respectively) is not considered to warrant or contribute to classification as the increase was not statistically significant and was not seen at higher doses (1 000 ppm or 2 500 ppm) in three other rat carcinogenicity studies.

Mouse carcinogenicity studies B.6.5.1/08 and B.6.5.1/09

No significant increases in tumour incidences were observed up to doses exceeding 1 000 mg/kg bw/d in study B.6.5.1/08. The other mouse carcinogenicity study (B.6.5.1/09) was also negative but the top dose level was too low (ca. 110 mg/kg bw/d, no effect on body weight and no other adverse effects). Overall, phenmedipham is not considered carcinogenic in the mouse.

Mode of action

A brief overview of genotoxicity studies is provided under 'Supplemental information' in the Background Document. The mutagenicity hazard class was not open for public consultation and was presented only as background information for carcinogenicity assessment in the CLH report. Phenmedipham was negative for point mutations and positive for chromosomal aberrations *in vitro*. An *in vivo* mouse micronucleus assay using a dose of 15 000 mg/kg bw was negative. RAC, in line with the DS, does not consider the available data to raise a significant concern about genotoxicity.

The DS proposed that some changes in the weight of reproductive organs seen in some studies could be used to support a hormonally mediated MoA of the pituitary and uterine tumours. However, it is not clear from the RAR whether there indeed was a consistent pattern of effects on the weights of reproductive organs across studies at doses not causing marked body weight reductions. As there is no robust MoA information, RAC retains the default assumption of human relevance of any observed tumours.

Conclusion on classification

A treatment-related increase in the incidence of pituitary adenoma was observed in one sex (male) of one species (rat). Although the tumour is benign, it can lead to adverse consequences by compressing the surrounding tissue or by excessive production of hormones. Pituitary adenoma has a relatively high background incidence in both rats and humans.

A treatment-related increase in benign tumours can in principle lead to classification in Category 2. However, taking into account the benign nature of the pituitary tumours, the high background incidence, the lack of preneoplastic lesions and occurrence in only one sex of one species, RAC concludes that **no classification for carcinogenicity** is warranted.

Supplemental information - In depth analyses by RAC

Overview of genotoxicity studies

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

Type of study	Reference (RAR); year	Result	Limitations
In vitro			
Ames	B.6.4.1/01; 1987	Negative	TA102 or *E.coli* WP2 uvrA not tested
Ames	B.6.4.1/02; 1987	Negative	TA102 or *E.coli* WP2 uvrA not tested
Ames	B.6.4.1/03; 2014	Negative	
Ames	B.6.4.1/04; 2016	Negative	
Chromosomal aberrations	B.6.4.1/05; 1994	Positive ±S9 at cytotoxic concentrations	
Chromosomal aberrations	B.6.4.1/06; 1986	Positive ±S9; marked increases in aberrations, +S9 only at a cytotoxic concentration	
Chromosomal aberrations	B.6.4.1/07; 2016	Positive –S9, cytotoxicity not excessive (MI 70 %)	
HPGRT	B.6.4.1/08; 1987	Negative	
Mouse lymphoma assay	B.6.4.1/09; 1986	Negative	Exposure for 2 hours (usually at least 3 h)

HPRT	B.6.4.1/10; 2016	Negative	
UDS	B.6.4.1/11; 1988	Negative	
In vivo			
Micronucleus (bone marrow, mouse)	B.6.4.2/01; 1985	Negative; dose 15 000 mg/kg bw	Limited reporting; bone marrow exposure not demonstrated within the study
Micronucleus (bone marrow, mouse)	B.6.4.2/02; 1978	Negative; top dose 1 000 mg/kg bw	Low top dose; limited reporting
Micronucleus (bone marrow, mouse)	B.6.4.2/03; 2017	Negative; top dose 2 000 mg/kg bw	Bone marrow exposure was low (plasma analysis, autoradiography)
Chromosomal aberrations (germ cells, mouse)	B.6.4.3/01; 1987	Negative; dose 15 000 mg/kg bw	

10.11 Reproductive toxicity

10.11.1 Adverse effects on sexual function and fertility

Table 15: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results				Reference
		Maternal and paternal NOAEL	Developmental NOAEL	Reproductive NOAEL	Effects	
Two generation study equivalent OECD 416 (1983) according to the principles of GLP Sprague-Dawley CD rat 24/sex/group see text for deviations <u>Acceptable (toxicity at highest dose appears to be low)</u>	phenmedipham, purity: 98.5% 0, 60, 250, 1000 ppm (M: 0, 4.5, 19, 76 mg/kg bw/day; F: 0, 5.2, 22, 86 mg/kg bw/day) Continuous in diet through 10 weeks premating, mating, gestation and lacatation periods	Maternal: 250 ppm/22 mg/kg bw/day Paternal: 1000 ppm/76 mg/kg bw/day	1000 ppm/76 mg/kg bw/day	1000 ppm/76 mg/kg bw/day	<u>Maternal:</u> Reduced body weight gain and food consumption <u>Pups:</u> None (minor decreases in body weight/body weight gain) <u>Reproduction:</u> None	1987 RAR B.6.6.1/01 M-146359-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**				**Reference**
		Maternal and paternal NOAEL	**Developmental NOAEL**	**Reproductive NOAEL**	**Effects**	
Two generation study equivalent OECD 416 (1983) according to the principles of GLP Wistar rat 24/sex/group see text for deviations <u>Acceptable</u>	phenmedipham, purity: 97±1% 0, 25, 75 and 225 mg/kg bw/day Continuous in diet through 10 weeks premating, mating, gestation and lacatation periods	Maternal: <25 mg/kg bw/day Paternal: 75 mg/kg bw/day	25 mg/kg bw/ day	225 mg/kg bw/ day	<u>Maternal:</u> Reduced body weight/body weight gain <u>Paternal:</u> Reduced body weight/body weight gain and reduced food consumption <u>Pups:</u> reduced body weight (both sexes) <u>Reproduction:</u> None	1986 RAR B.6.6.1/02 M-146382-01-1 and a 3rd addendum to the report: 2000 M-238764-01-1
Three generation study Sprague-Dawley CD rat in-house method, according to the principles of GLP (see RAR for details) <u>Supplemantary study only</u> due to too low doses	phenmedipham, purity unknown 0, 20, 100 and 500 ppm (M: 0, 1.3, 6.6, 35 mg/kg bw/day; F: 0, 1.7, 8.1, 43 mg/kg bw/day) Continuous in diet	500 ppm/35 mg/kg bw/day	500 ppm/35 mg/kg bw/day	500 ppm/35 mg/kg bw/day	<u>Parents:</u> None <u>Pups:</u> None (minor decreases in body weight were observed) <u>Reproduction:</u> None	1979 RAR B.6.6.1/03 M145588-01-1

Table 16: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	**Test substance,**	**Results**	**Reference**

Type of study/data	Test substance,	Results	Reference
24-month oral carcinogenicity study in rats OECD 453, GLP Rat, Sprague-Dawley 50/sex/group	phenmedipham batch no. JS148 Purity: 98.5% 0, 60, 250, 1000 ppm M: 3, 13, 50 mg/kg bw/day F: 4, 17, 68 mg/kg bw/day	There were no remarkable findings in histopathology of testes and epididymis.	1988b, 2000 RAR B. 6.5.1/03-04 M-146366-02-, M-199354-01-1
24-month oral carcinogenicity study in rats, OECD 453, GLP Rat, Sprague-Dawley 50/sex/group	phenmedipham Purity: 97±1% 0, 60, 250,1000 ppm M: 3, 14, 55 mg/kg bw/ day F: 4, 18, 73 mg/kg bw/ day	There were no remarkable findings in histopathology of testes and epididymis.	1988c RAR B.6.5.1/05 M-146387-03-1
24-month oral toxicity/ carcinogenicity study in rats Rat, Han Wistar 70/sex/group	phenmedipham 0, 100, 500, 2500 ppm M: 4.6, 23.6, 117.6 mg/ kg bw/day F: 6.4, 33.1, 170.5 mg/ kg bw/day	Statistically significant decrease in testes seminiferous tubular atrophy at 500 and 2500 ppm. Significant decreases in incidences of absent spermatozoa and degenerate spermatogonic ducts in epididymis at 2500 ppm.	2004 RAR B.6.5.1/07 M-240148-01-1
24-month oral toxicity/ carcinogenicity study in rats, OECD 453, GLP Rat, Sprague-Dawley, Charles River CD 60/sex/group	phenmedipham Lot No. U.S.A.- 7038, Batch No. 260-121 Purity: assumed 100% 0, 20, 100, 500 ppm M: 1, 5, 28 mg/kg bw/ day F: 1, 7, 34 mg/kg bw/ day	According to individual histopathology data aspermatogenesis seems to be slightly increased at 500 ppm compared to control group in terminally sacrificed animals (incidences 9.7% and 23.6% at 0 and 500 ppm, respectively), but no definite conclusion can be drawn due to poor reporting.	1980 RAR B.6.5.1/06 M-145589-01-1
78-week dietary carcinogenicity study in CD-1 mice, , GLP, OECD 451	phenmedipham 0, 500, 2000, 7000 ppm M: 82, 331, 1167 mg/ kg bw/day F: 107, 443, 1532 mg/ kg bw/day	There were no remarkable findings in histopathology of testes.	1991 RAR B.6.5.1/08 M-146395-01-1
104 week dietary carcinogenicity study in CD-1 mice, GLP	phenmedipham 0, 10, 100, 1000 ppm M: 1.1, 11, 110 mg/kg bw/day	There were no remarkable findings in histopathology of testes.	1987 RAR B.6.5.1/09 M-145666-01-1

Type of study/data	Test substance,	Results	Reference
	F: 1.2, 12, 117 mg/kg bw/day		
Repeated dose toxicity studies reviewed in chapter 10.13.		When examined, there were no remarkable findings in histopathology of testes and epididymis in these studies.	RAR B.6.3.1 and 6.3.2

For futher details on studies see chapters 10.9 and 10.13 of this report and RAR.

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

Two two generation studies and one combined reproduction and teratogenicity study (three generation study) are available for phenmedipham (Table 15). The two generation studies (**RAR B.6.6.1/01-02**) generally comply with an older OECD 416 guideline (1983) and therefore a range of sensitive endpoints included in the 2001 updated guideline were not investigated (e.g. estrous cycle length and normality, no. corpora lutea, various sperm parameters, age of sexual maturation of offspring and various organ weights including reproductive organs). The three generation study (**RAR B.6.6.1/03**) is only considered supplementary since no adverse effects were observed due to too low doses. The studies are only described briefly in the text below. Further details on studies including deviations from the guidelines are given in RAR.

In the first two generation study (**RAR B.6.6.1/01**) Sprague Dawley CD rats were treated with phenmedipham doses 0, 60, 250, and 1000 ppm via diet corresponding to 0, 4.5, 19 and 76 mg/kg bw/day in males and 0, 5.2, 22 and 86 mg/kg bw/day in females, respectively.

The main deviations from the guideline (OECD 416, 1983/2001) were the following. Body weights of F0 animals were only recorded one week prior to treatment, thereafter weekly until the start of the mating period, and at termination. Post-weaning F1 animals were weighed weekly from selection until the start of their mating period, and then at termination. Likewise food consumption of parental animals was only recorded until start of the mating period. Pre-weaning F1 and F2 pups were weighed individually only on day 21 of lactation. On days 1, 4 and 12 of lactation the pups were weighed by the litter (en masse) sexes separately. The number of corpora lutea was not reported and thus effects on preimplantation loss can not be assessed. Organ weights and histopathology of organs including reproductive orgarns were not reported. The statistical analyses are poorly reported and it is unclear whether covariates were considered (e.g. no. pups/litter) when appropriate.

There were no mortalities or remarkable clinical signs or necropsy findings in parental animals. There were no differences in body weights or body weight gains between the treated parental male groups and controls. Since body weights of dams were not recorded during the gestation and lactation periods, maternal toxicity in this study is difficult to evaluate. However, high dose (86 mg/kg bw/day) F0 female body weight gains during premating period (weeks 0-10) and terminal body weights were statistically significantly reduced compared to controls (14 % and 6.6% decreases in body weight gain and body weight, respectively). Food consumption of high dose F0 animals was consistently slightly, but not statistically significantly reduced compared to controls (9.5.% decrese in females). Body weights and body weight gains (weeks 5-13) of F1 females decreased in a dose-related manner and were statistically significantly reduced compared to controls in high dose group (10.4% and 15.0%, decreases, respectively). The weight reduction in high dose F1 females persisted over weeks 5-13. Food consumption of mid (22 mg/kg bw/day) and high dose (86 mg/kg bw/day) F1 animals was slightly, but not statistically significantly reduced compared to controls (4.% and 8.8% reductions in high dose males and females compared to controls, respectively). We note that general toxicity at the highest dose tested appears to be low. Thus it may be argued whether the dosing in the study was appropriate.

The parameters of mating and gestation (gestation length, gestation index), were comparable between the phenmedipham treated groups and the controls in both generations. The fertility indexes of F0 control (67%) and F0 low dose groups (71%) were unusually low leading to low number of dams with viable litters in these groups (Table 17). However, there were no relation of fertility and phenmedipham treatment in either

generation. There was slightly higher incidence of inadequate maternal care in the phenmedipham treated groups in both generations (Table 17). Moreover, survival of F1 pups on day 4 of lactation (viability index) and on day 21 of lactation i.e the number of pups weaned were slightly decreased at mid (22 mg/kg bw/day) and high doses (86 mg/kg bw/day, Table 17). Similar effect on early viability of pups was not observed in F2 generation and the difference in the final number of pups weaned between the control and mid and high dose groups was smaller (Table 17). No clinical signs or necropsy findings attributable to treatment were reported in pre-weaning pups. Mean body weights and body weight gains of high dose (86 mg/kg bw/day) litters of both generations were slightly reduced compared to controls throughout the lactation period (RAR and Table 18). The reductions were not statistically significant, but it is noted that litter size was apparently not used as covariate in the analyses.

Table 17: Reproductive performance and maternal care in the two generation study (RAR B.6.6.1/01)

F1 generation	**phenmedipham (mg/kg bw/day)**			
	0	**4.5**	**22**	**86**
No. of pregnant females (F0)	16	17	23	23
Fertility index (%) [e]	67	71	96	96
No. dams with viable litters	16	17	23	23
Incidence of deficiency in maternal care[a]	2 (13%)	2 (12%)	4 (17%)	6 (26%)
No. live pups at birth (mean±s.d)	12.8±4.2	14.1±2.1	13.0±3.4	12.6±3.6
No. dead pups at birth (mean±s.d)	0.19±0.40	0.18±0.53	1.43±4.07	0.26±0.69
Live birth index[b] (mean/median)	91.9% 100	95.8% 100	93.9% 100	99.7% 100
Viability index days 0-4 [c] (mean/median)	86% 100.0	92.5% 100	78.9% 92.9	73.7% 92.9
Lactation index days 4-21[d] (mean/median)	93.5% 100	99.1% 100	92.0% 100	93.7% 100
No. weaned (live at day 21 of lactation), (mean±s.d)	10.4±4.9	12.7±3.3	9.3±5.0	8.8±5.5
No. weaned as % of live pups at birth	81%	90%	72%	70%
F2 generation				
No. of pregnant females (F1)	24	21	22	24
Fertility index (%)	100	88	92	100
No. dams with viable litters	24	21	22	24
Incidence of deficiency in maternal care[a]	5 (21%)	5 (24%)	7 (32%)	6 (25%)
No. live pups at birth (mean±s.d)	12.0±3.5	12.2±3.1	11.7±2.0	11.8±2.4

No. dead pups at birth (mean±s.d)	1.67±3.76	1.19±2.91	0.86±1.08	0.04±0.20
Live birth index[b] (mean/median)	91.9% 100	95.8% 100	93.9% 100	99.7% 100
Viability index[c] days 0-4 (mean, median)	73.4% 90.9	74.2% 92.9	70.2% 92.0	73.9% 100
Lactation index[d] days 4-21[d] (mean/median)	93.5% 100	99.1% 100	92.0% 100	93.7% 100
No. weaned live at day 21 of lactation (mean±s.d)	9.0±5.1	8.8±5.4	7.9±5.2	8.1±5.0
No. weaned as % of live pups at birth	75%	72%	68%	69%

[a] maternal care: construction and cleaning of the nest, whether the pups were kept together, clean and warm and not left scattered, and whether the pups were physically abused.

[b] No. pups live on day 0 / no. pups born, [c] No. pups live on day 4 / live on day 0, [d] No. pups live on day 21 / no. live on day 4

[e] No. animals paired / no. pregnant

Table 18: Mean body weight gain changes of high dose litters in the two generation study (RAR B.6.6.1/01)

Mean body weight change of pups (% of control)	phenmedipham (86 mg/kg bw/day)			
	F1 generation		F2 generation	
Lactation day	males	females	males	females
1	-6.1	-6.6	-1.6	-1.7
4	-4.2	-5.7	-4.3	-3.3
12	-3.8	-6.2	-6.6	-8.5
21	-3.8	-8.3	-7.5	-7.7
Mean bw gain (day 1-21, % of control)	-3.4	-8.6	-8.4	-8.5

The individual animal data reveals causal association between inadequate maternal care and early mortality of pups in both generations in this study. Most of the dams reported to care their pups inadequately regardless of the treatment group, loosed their whole litter by the day 12 of lactation (F0 dams 8/14, 57% and F1 dams 18/23, 78%). For example in F1 generation 4 out of 5 control dams caring deficiently loosed their whole litter and 5 out of 6 high dose dams caring decifiently loosed their whole litter. No whole litter mortalities during the lactation period were reported for dams that cared their pups adequately. There were no remarkable differences in pup mortality between the control dams and the treated dams, that were reported to care their pups adequately. We note that in F1 generation the difference in maternal care between control and the treated groups is very slight and therefore the effect on early survival of pups seems to diminish. We further note that low number of control and low dose dams with viable litters in F0 generation (16 and 17, respectively) may have skewed the results of F0 generation (the incidence of deficient maternal care of F1 generation control dams was 21% compared to 15% in F0 generation). According to study report both maternal care and pup viability have varied in the laboratory within the ranges observed in this study without relationship to treatment.

We conclude that it remains obscure whether slightly increased incidence of inadequate maternal care and slightly increased early mortality of pups are treatment-related. These findings did not reach statistical significance and the effect on early mortality was not repeated in F2 generation. The biological significance of these findings and the effect of phenmedipham on pup body weights are considered equivocal.

In the other two generation study higher dietary doses of phenmedipham, corresponding to 0, 25, 75 and 225 mg/kg bw/day were used (**RAR B.6.6.1/02**). The main deviations from the guideline (OECD 416, 1983/2001) were the following. Food consumption of parental animals was only recorded during pre-mating period. Individual pup body weights were only recorded on day 4 for the selected animals, on days 1, 4 12, 21 of lactation the pups were weighed by the litter (en masse) sexes separately. Organ weights were not reported. Numbers of corpora lutea and implantantion sites were not reported. Moreover, inappropriate statistical analyses were used, i.e. Student´s t-test for multiple comparisons. It also appears as if analyses did not use litter as the unit when relevant and appropriate covariates (e.g. no. pups/litter).
Parental toxicity was evident in this study, mainly manifested as reductions in body weight performances in both generations. In F0 generation mean body weights of F0 males were statistically significantly reduced at the end of dosing (7% decrease compared to control at day 69) and mean body weights of high dose females were statistically significantly reduced throughout the premaiting period (6 % decrease compared to controls at day 69). The body weight gains of high dose (225 mg/kg bw/day) animals were reduced by 14% and 8%, in females and males compared to controls, respectively. During pre-mating period food consumption of high dose F0 males was statistically significantly reduced compared to controls (by 7%) and efficiency of food utilization was slightly but dose-dependently reduced in phenmedipham treated F0 females. During the

gestation and lactation periods mean body weights of high dose F0 dams were statististically significantly lower than the controls (9% decrease on lactation day 21). Body weight gain of high dose F0 dams was reduced by 34% on days 1-21 of lactation, but this difference was not statistically significant. Phenmedipham treated F1 females had statistically significantly lower body weights compared to controls starting from the premaiting period until end of the study (day 69). Moreover, the mean body weights of high dose F1 dams were statistically significantly reduced throughout the gestation and lactation periods (8-10% and 13%, over gestation and lactation days 1-21, respectively) and mean body weight gains of all treated groups were dose-dependently and statistically significantly reduced compared to controls during lactation period (by 44%, 50% and 69% in low, mid and high dose groups, respectively, Table 19).

Table 19: Mean body weights of F1 females during gestation and lactation (g ± s.d.)

Gestation day	**phenmedipham (mg/kg bw/day)**			
	0	**25**	**75**	**225**
0	219.3±12.5	209.3* ±14.4	211.0 ±13.7	197.7± 12.8**
7	239.4±12.9	229.7±17.2	230.3±13.1	216.5±15.1**
14	263.8±16.0	257.1±19.1	254.8±15.2	241.3±17.2**
20	313.2±26.5	310.3±24.3	306.0±22.9	287.0±27.0**
Body weight gain (days 0-20)	93.9±22.3	101.0±13.4	95.5±14.6	89.3±21.6
Lactation day	**0**	**25**	**75**	**225**
1	246.3±15.7	241.6±20.2	241.3±16.0	225.1±17.8**
4	251.4±17.2	245.5±20.4	243.7±15.2	226.9±14.7**
7	251.9±14.3	245.0±18.2	245.3±15.3	228.5±18.4**
14	272.2±21.5	264.6±20.6	261.4±15.6	238.0 ±17.4**
21	267.3±12.5	253.4±21.0*	251.8±15.8**	231.3±17.8**
Body weight gain (days 1-21)	20.9±12.2	11.8±10.0**	10.4±8.6**	6.5±9.3**

*statistically significant difference to control P<0.05, ** p <0.01

No treatment related clinical signs, mortalities or necropsy findings were reported in parental animals. The following organs of control and high dose (225 mg/kg bw/day) parental animals (including few low and intermediate dose animals) were examined histopathologically: ovaries, uterus, cervix, vagina, testes, epididymis, seminal vesicles, prostate, coagulating gland, pituitary gland. The examinations revealed no treatment related degenerative, inflammatory or neoplastic changes in these tissues. According to study report testis showed normal spermiogenesis and ovaries normal number of maturing follicels and corpora lutea in various stages of involution. The parameters of mating and gestation were comparable between the phenmedipham treated groups and the controls in both generations. In F0 generation fertility and pregnancy rates were comparable in treated groups and in controls (fertility 96%, 83%, 96% and 92%, pregnancy 88%, 83%, 92% and 88% at 0, 25, 75 and 225 mg/kg bw/day, respectively). In F1 generation the fertility rates were high in all groups (96-100%) and the pregnancy rate was only slightly lower in the high dose group compared to other groups (96%, 100%, 100% and 92% at 0, 25, 75 and 225mg/kg bw/day, respectively). The numbers of implantation sites per dam were comparable between the groups in both generations.

No clinical signs or necropsy findings attributable to treatment were reported in pre-weaning pups. No remarkable differences in litter size, sex ratio, pup mortality, malformations and numbers of pups during the lactation period between the groups were observed in either generation. Adequancy of maternal care was not reported in the study report. The group mean body weights of high dose (225 mg/kg bw/day) F0 generation pups (both sexes) were statistically significantly reduced compared to controls on lactation day 21 (9% and 10% in males and females, respectively, RAR). On lactation day 14 body weights of F0 high dose female pups were significantly reduced compared to control pups (7 %).

Table 20: Group mean body weights (g ± s.d.) of F1 litters (F2 generation) during lactation

Males Lactation day	**phenmedipham (mg/kg bw/day)**			
	0	**25**	**75**	**225**
No. pups	22	23	24	22
1	6.582 ± 0.6205	6.657 ± 0.654	6.425 ± 0.516	6.505 ± 0.727
4	9.52 ± 1.20	9.64 ± 1.38	9.26 ± 1.36	9.20 ± 1.09
7	14.46 ± 1.53	14.55 ± 1.73	13.95 ± 1.75	13.54 ± 1.88
14	28.00 ± 2.56	27.65 ± 2.25	26.45 ± 2.90	23.80 ± 3.62*
21	43.52 ± 3.66	41.54 ± 4.68	39.60 ± 5.06*	35.36 ± 5.47*
Females Lactation day	**0**	**25**	**75**	**225**
No. pups	23	23	24	22
1	6.322 ± 0.687	6.283 ± 0.697	6.037 ± 0.646	6.309 ± 0.878
4	9.40±1.58	9.13 ± 1.39	8.78 ± 1.26	9.10 ± 1.52
7	14.33 ± 2.14	14.17±1.65	13.20 ± 1.63*	13.25 ± 2.05
14	27.60±3.45	27.34±2.10	25.40 ± 2.56*	23.45 ± 3.75*
21	42.35±4.62	40.69±4.11	37.87 ± 4.12*	34.98 ± 5.73*

*statistically significant difference to control P<0.05

In F2 generation the group mean body weights for male pups in the intermediate dose group at lactation day 21 and high dose group at days 14 and 21 were significantly lower than in controls (by 9%, 15% and 19%, respectively, Table 20). The group mean body weights for female pups in the middle dose group at days 7, 14 and 21 and high dose group at days 14 and 21 were significantly lower than in controls (by 8%, 8%, 11%, 15% and 17%, respectively, Table 20).

Pups were not weighed individually in this study and it is difficult to assess the link between maternal toxicity and decreased group pup weights at mid and high doses based on the individual animal data. However, in repeated dose toxicity studies (chapter 10.13) haemotological effects have been reported at these dose levels. Therefore we conclude that the observed effect of phenmedipham on pup body weights accentuated over generations at 75 and 225 mg/kg bw/day is plausibly a secondary non-specific consequence of other toxic effects.

The three generation reproduction and teratology study in rats (**RAR B.6.6.1/03, 1979**) was performed as an in-house method. The main deviations from the OECD TG 416/414 and other shortcomings include e.g. the following: individual pup body weights and weights of parental females during gestation and lactation were not determined, gestation lengths and organ weights were not determined, histopathology of selected organs was not conducted on parental animals, initial body weights of parental animals deviated more than ± 20% of the mean, only 15 females/group were exposed, inappropriate statistical analyses were used. Finally, since the doses used (0, 20, 100 and 500 ppm corresponding to 0, 1.3, 6.6, 35 and 0, 1.7, 8.1, 43 mg/kg bw/day, in males and females, respectively) did not result any adverse effects, the study is regarded supplementary only. Further details on this study are given in RAR.

Table 16 summaries other studies relevant for toxicity on reproduction and sexual function. No remarkable histopathological findings in testes or epididymis were reported in repeated dose toxicity studies (**RAR B. 6.3)** with phenmedipham**.** In one chronic toxicity/carcinogenicity study in rats (**RAR B.6.5.1/07, 2004**) a dose-related reduction in testes seminiferous tubular atrophy was observed that was statistically significant in

the mid and high dose groups [10/50 (0), 6/50 (100), 2/50 – p<0.05 (500), 0/50 - p<0.01 (2500)]. In epididymides of terminal kill males, the incidence of 'spermatozoa absent' and 'degenerate spermatogenic cells in duct(s)' was dose-dependently reduced and became statistically significant in the high dose group [5/41 (0), 4/42 (100), 1/40 (500), 0/43 - p<0.05 (2500) and 7/41 (0), 5/42 (100), 2/40 (500), 1/43 - p<0.05 (2500), respectively]; the findings in epididymides were not observed in any of the prematurely killed males, however, in all animals combined, the reductions did not reach statistical significance. In RAR it is considered that these findings may reflect hormonal changes and specifically a dose-related decrease in age-related atrophy may be causally linked to the dose-related increase in pituitary tumours seen in males in the same study. We conclude that while this finding may be related to hormonal changes, decrease in age-related seminiferous tubular atrophy does not warrant classification for fertility effects.

According to individual animal histopathology data of another rat two-year carcinogenicity study (**RAR B.6.5.1/06, 1980**) aspermatogenesis seemed to be slightly increased at 500 ppm compared to control group in terminally sacrificed animals (incidences 9.7% and 23.6% at 0 and 500 ppm, respectively). Aspermatogenesis was reported in a few decedents animals of other groups including the control group but not in the high dose group decedents (the total number of animals examined per group remains unclear). Unfortunately, histological non-neoplastic findings were not summarised in tabular form in this study and the individual animal recordings do not reveal the number of low and mid dose animals examined histopatologically. We conclude that due to poor reporting and other deficiencies in the study no conclusion can be drawn from these findings.
No remarkable histological findings in testes and epididymis were reported in the remaining two rat carcinogenicity studies available for phenmedipham **(RAR 6.5.1/03, 1988b, 6.5.1/05, 1988c)**. Nor did the mice carcinogenicity studies with phenmedipham report any remarkable findings in testes histopathology **(RAR 6.5.1/08, 1991, 6.5.1/09, 1987)**.

10.11.3 Comparison with the CLP criteria

Phenmedipham had no effects on sexual function and fertility in rat generational studies. Slight increase in aspermatogenesis was reported in one rat carcinogenicity study but no similar findings were observed in other chronic toxicity or repeated dose toxicity studies with phenmedipham. Moreover, due to poor reporting of the study no conclusion can be drawn based on this finding. Therefore, the data presently available on phenmedipham does not warrant classification for fertility.
We note that in PPP assesment data on reproduction was considered insufficient to conclude on this endpoint (data gap, see RAR B.6.6). It could be also argued whether the data is sufficient to conclude on classification for fertility.

10.11.4 Adverse effects on development

Table 21: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results			Reference
		Maternal NOAEL	Developmental NOAEL	Effects	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**			**Reference**
		Maternal NOAEL	**Developmental NOAEL**	**Effects**	
Teratogenicity OECD 414 (1981), according to the principles of GLP Rat, Wistar 22 females/group Numbers of live and dead fetuses are not reported <u>Supportive study only</u>, as the purity of one out of two batches of test material used is unknown.	phenmedipham, purity: 97±1% 0, 516, 1160, 2580 mg/kg bw/day oral (gavage) days 6-15 of gestation	<516	<516	<u>Maternal:</u> Reduced (corrected) BW gain and food consumption <u>Pups:</u> Reduced body weight and incomplete ossification of neck vertebrae at all doses	1989 RAR B.6.6.2/01 M-146392-01-1
Preliminary limit test of embryotoxicity (including teratogenicity) in rat Rat, Wistar/HAN 5 females/group	phenmedipham technical, purity: 97.8%, 0 and 1000 mg/kg bw/day oral (gavage) days 6-15 of gestation			<u>Pups</u> Runts were observed in treated group but not in the control group	1988 RAR B.6.6.2/02 M-145777-01-1
Teratogenicity OECD 414 (1981), GLP Rat, Wistar/HAN 25 females/group Key study	phenmedipham technical, purity: 97.8%, 0, 150, 450, 1350 mg/kg bw/day oral (gavage) days 6-15 of gestation	<150	<150	<u>Maternal:</u> Reduced body weight gain <u>Pups:</u> Runts were observed in all treated groups but not in controls. Ossification effects in the absence of fetal body weight effects were observed at 1350 mg/kg bw/day.	1988 RAR B.6.6.2/02 M-145693-01-1
Teratogenicity OECD 414 (1981), GLP Rabbit, New Zealand White 16-21 females/group The doses were spaced too large intervals. Gravid uterine weights were not determined. The mean fetal	phenmedipham technical, purity: 99.3% 0, 5, 71, 1000 mg/kg bw/day oral (gavage) days 6-18 of gestation	71	1000	<u>Maternal:</u> Reduced body weight gain and food consumption <u>Pups:</u> None	1992 RAR B.6.6.2/03 M-145737-01-1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results			Reference
		Maternal NOAEL	Developmental NOAEL	Effects	
weights were not reported for each sex. The no. does/group is low.					
Teratogenicity OECD 414 (1981), GLP Rabbit, New Zealand White 15 females/group only 2/3 of the fetuses were examined for skeletal effects, unclear if the control group was an appropriate vehicle control group. The no. does/group is low.	0, 50, 225 and 1000 mg/kg bw/day phenmedipham technical, purity: 98.5% oral (gavage) days 6-18 of gestation	225	225	<u>Maternal:</u> Reduced (corrected) body weight gain and food consumption <u>Pups:</u> Reduced body weight and retarded ossification	1986 RAR B.6.6.2/04 M-146352-01-1

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

Two rat and two rabbit developmental toxicity studies are available for phenmedipham. The studies generally comply with and older OECD 414 guideline (1983) and therefore the dosing was during organogenesis only. Furthermore, the statistical analyses performed were generally not well described and the results appear not always to have been evaluated by an appropriate statistical method; it appears as if the litter was seldom used as the unit for data analysis when appropriate and likewise relevant covariates (e.g. number of pups per litter) were typically not included in the data analyses. The studies are only described briefly in the text below. Further details on studies including deviations from the guidelines are given in RAR.

The first rat developmental toxicity study (**RAR B.6.6.2/01, 1989**) is considered supportive only due to unknown purity of one out of two batches of test material used. In this study maternal toxicity was evident at all tested doses (0, 516, 1160, 2580 mg/kg bw/day orally by gavage) in the form of reduced food consumption, reduced maternal body weight gain during organogenesis and reduced total body weight gain corrected for uterus weight. The fetal body weights were significantly reduced in the mid (1160 mg/kg bw/day) and high dose (2580 mg/kg bw/day) groups and statistically non-significantly reduced in the low dose (516 mg/kg bw/day) group. Incomplete ossification of neck vertebrae of foetuses was statistically significantly increased at all tested doses. These ossification effects presumably reflect lower mean body weights of phenmedipham treated foetuses. In addition, there were some less remarkable skeletal findings that reached statistical significance (RAR Table B.6.6.2-7).

In the other rat developmental study (**RAR B.6.6.2/02, 1988**) lower doses were used; 0, 150, 450, 1350 mg/kg bw/day (orally by gavage). Maternal toxicity was evident at all doses manifested by reduced body weight gain (Table 22). Body weights of high dose (1350 mg/kg bw/day) dams were statistically significantly reduced on days 12, 16, 17 and 18 by 3.4-3.7% compared to controls. Food consumption was

significantly reduced in high dose dams compared to controls during days 6-11 (by 8.8%). Slight reductions in mean food consumption were also reported in low and mid dose groups at days 6-11.

Table 22: Mean maternal body weight gains (g) during gestation in rat teratogenicity study (RAR 6.6.2/02, 1988)

Day of gestation	Dose (mg/kg bw/day)			
	0	150	450	1350
0-6	17	16	18	18
6-11	18	14 (22% ↓)	14 (22 %↓)	11 (39 %↓)
6-16	43	38 (12% ↓)	35 (19 %↓)	34 (21 %↓)
6-21	91	84 (7.7% ↓)	85 (6.6 %↓)	82 (9.9 %↓)
6-21#	14.6±8.0	15.1±9.8	12.1±13.6	9.7±8.6
6-21##	6.6	6.9 (5%↑)	5.5 (17 %↓)	4.2 (36 %↓)

Corrected body weight gain (uterus weight excluded) ## corrected body weight gain % of body weight on day 6. It is unclear whether body weight gains were evaluated statistically.

Slight not statistically significant increases in embryonic and fetal resorptions and post implantation losses were observed in all phenmedipham treated groups compared to controls (Table 23, RAR). We note that one dam of the control group had total resorption and was excluded from the analyses (only dams with viable fetuses at termination were included). Moreover, when the data is compared with historical control data generated 1984-1986 in the laboratory (the study was conducted November 1987-December 1987), it appears that the mean value per dam of embryonic resorptions (**0.4**) and the mean percentage post implantation loss for the concurrent control (**3.3%**) were lower than the mean HC values (vehicle treated controls **0.71 and 6.1%**), whereas the mean values for the treated groups were comparable to those of the HCD (RAR). This suggests that the differences between treated groups and the concurrent control group are not treatment related.

Table 23: Summary of reproduction parameters in rat teratogenicity study (RAR 6.6.2/02, 1988)

	phenmedipham (mg/kg bw/day)			
	0	150	450	1350
No. of pregnant females	25	23	21	25
No. dams with viable fetuses at termination	24#	23	21	25
Mean no. of implantations ± s.d /dam	12.6 ± 1.6	11.6 ± 2.2	12.2 ±2.2	12.5 ± 1.4
No. post-implantation loss / group	10	16	15	23
Mean no. post implantation loss / dam	0.4	0.7	0.7	0.9
Post implantation loss (%)	3.3	6.0	5.9	7.4
No. foetuses ##	293	250	241	289
Mean no. fetuses ± s.d /dam	12.2 ± 1.4	10.9 ±2.5	11.5 ±2.6	11.6 ±1.6
No. male fetuses (%)	136 (46.4%)	117 (46.8%)	118 (49%)	149 (51.6%)
No. female fetuses (%)	157 (53.6%)	133 (53.2%)	123 (51%)	140 (48.4%)*

Mean fetal body weight**				
males ± s.d (g)	4.7 ± 0.2	4.7 ± 0.3	4.9 ± 0.3	4.7 ± 0.3
females ± s.d (g)	4.5 ± 0.2	4.5 ± 0.3	4.5 ± 0.3	4.5 ± 0.3

One female had total resorption and was excluded from calculations of reproduction data, ## All fetuses were viable
*Statistically significant difference to control at P < 0.05, Fischer´s Exact test, ** unweighted mean of litter means and variation between litters

The proportion of female fetuses was significantly reduced in the high dose group compared to control group (**48.4% and 53.7%** at high dose and controls, respectively, p<0.05, Table 23). In the study report the high dose group mean female ratio of **48.4%** is claimed to be within the historical control range of the laboratory, **45.4.-51.8%** (1984-1986, means 50.2% of 5280 pups and 51.7% of 362 pups for vehicle-treated and untreated controls, respectively). In the preliminary limit study (dose-range finding study) conducted in the same laboratory the sex ratio was changed in the opposite direction (Table 24).

Table 24: Sex ratio of fetuses in the pilot study

Sex ratio of fetuses	Dose (mg/kg bw)	
	0 (n=41)	1000 (n=54)
Males (%)	46.3	40.7
Females (%)	53.7	59.3

However, in the main study mean female ratios of the control and low dose groups were approximately the same (53.6% and 53.2%), whereas the female ratios at mid and high dose groups were lower (51% and 48.5%). Moreover, since there were significantly lower number of fetuses in the preliminary limit study, we consider that the divergence in sex ratio of these studies does not overrule the findings of the main study. Therefore, we conclude that it can not be excluded that the changed sex ratio is treatment-related.

Malformations in the form of runts were observed at all doses of phenmedipham. External examination found four runts: one in the low (male, 2.5g; umbilical hernia noted at visceral examination but not present at fresh fetal examination) and in the mid (female, 2.2g) dose group and two in the high dose group (females, 2.4 and 2.6g, same litter). No runts were found in the concurrent control group (one small female fetus with brachygnathia was reported, 3.6g). No further abnormal fetuses were detected in visceral examination but control fetus with brachygnathia at external examination was found to have a partial twin. In the HCD from the performing laboratory in 1985, only three runts were reported out of 2314 fetuses (gavage-treated controls) and 2908 fetuses (inactive treatment) examined and HCD from 1986 did not report any runts amongst 1381 fetuses (gavage-treated controls) and 1103 fetuses (inactive treatment) examined. The in-life phase of the study (**RAR B.6.6.2/02, 1988**) was conducted over 11/1987-12/1987.

Table 25 summarizes external fetal findings and individual data of dams that gave birth to abnormal fetuses. We note that at intermediate and high doses runts were born for dams with litter sizes greater than mean values and the corrected body weight gain of the high dose dam was negative. The corrected body weight gains of low and intermediate dose dams that gave birth to runts were higher than mean values. The small fetus with brachygnathia was born to control dam with litter size greater than average and corrected body weight gain lower than mean of the control group. In a preliminary range finding study where 5 pregnant females were exposed to 1000 mg/kg bw/day of phenmedipham there were two runts in one litter in the phenmedipham-treated group (one male of 2.6 g and one female of 3.0 g, both with hydrocephaly and the male also had brachygnathia), whereas no runts were seen in the four control litters (fetus incidence 3.7%, litter incidence 20%). The litter size of the dam that gave birth to runts was smaller than average (8 pups compared to mean number of 10.8) and the dam had higher corrected body weight gain than mean value of the group (35 g compared to mean value of 20.8 g).

We conclude that occurrence of runts in phenmedipham treated groups may be treatment-related.

Table 25: Summary of external fetal findings in rat teratogenicity study (RAR B.6.6.2/02, 1988)

	phenmedipham (mg/kg bw/day)				
	0		**150**	**450**	**1350**
External foetal findings					
No. litters examined	24		23	21	25
No. fetuses examined	293		250	241	289
Runt (% of fetuses; % of litters)	0		1 (0.40;4.3)	1 (0.41; 4.8)	2 (0.69; 4)
Small foetus (3.6 g) with brachygnathia	1		0	0	0
Missing tail	1		0	0	0
Dam identity	7*	16**	33	68	76***
Litter size	14	13	7	12	14
Body weight gain 6-21	95	99	74	90	73
Uterus weight (g)	90	81	42	71	74
Corrected body weight gain 6-21 (g)	5	18	32	19	-1
Corrected body weight gain 6-21 (%)	2.1	8.1	14.9	9.3	-0.4

*Dam no. 7 gave birth to small female foetus (3.6 g) with brachygnathia, **Dam no.16 gave birth to foetus with no tail, ***Dam no. 76 gave birth to two runts

In skeletal examination a significant decrease in the incidence of non-ossified metatarsalia 1 of the left hind limb was observed in high dose group, and similar findings were seen for the right hind limb. This decrease appears to be dose-dependent (Table 26). In addition, a statistically significant decrease in incompletely ossified sternebra 6 was observed for all phenmedipham-treated groups. However, the finding did not show any dose-dependency and a similar pattern was not observed for sternebra 1-5 (RAR). A statistically significant increase in abnormally ossified sternebra 4 was observed for fetuses in the high dose group and a similar but statistically non-significant increase was seen for litters. Significant findings of abnormal ossification of the other sternebra were not observed. Finally, statistically non-significant observations of incompletely ossified cervical vertebra (1, 2, 4 and 5) were reported for the high-dose group (Table 26).

These above-mentioned ossification effects were observed in association with low to moderate maternal toxicity. However, since also enhancement of ossification in phenmedipham treated groups compared to concurrent controls is reported (e.g. matatarsalia), they do not simply imply a general delayed ossification. Therefore, we conclude that these skeletal findings cause additional concern for developmental toxicity of phenmedipham.

Table 26: Selected skeletal findings in rat teratogenicity study (RAR B.6.2.2/02, 1988)

	phenmedipham (mg/kg bw/day)			
	0	**150**	**450**	**1350**
Skeletal findings				
No. fetuses examined	147	126	120	144
No. litters examined	24	23	21	25
Left hindlimb, non-ossified metatarsalia 1 No. foetuses (%)	42 (29%)	48 (38%)	25 (21%)	22 (15%)**

No. litters (%)	18 (75%)	16 (70%)	11 (52%)	12 (48%)*
Right hindlimb, non-ossified metatarsalia 1				
No. foetuses (%)	37 (25%)	46 (37%)*	24 (20%)	22 (15%)*
No. litters (%)	18 (75%)	17 (74%)	11 (52%	12 (48%)*
Sternum, incompletely ossified sternebra 6				
no. foetuses	34 (23%)	17 (13%)*	15 (13%)*	19 (13%)*
no. litters	20 (83%)	10 (43%)**	7 (33%)**	12 (48%)*
abnormally ossified sternebra 4				
no. foetuses	0	1 (1%)	0	6 (4%)*
no. litters	0	1 (4%)	0	4 (16%)
Cervical vertebra, incompletely ossified (no. foetuses/no. litters)				
cervival vertebrae 1	0	0	0	2/2
cervival vertebrae 2	0	0	0	1
cervival vertebrae 4	0	0	0	1
cervival vertebrae 5	0	0	0	1

*Statistically different from control at P<0.05, **P<0.01, Fischer exact test

In the first developmental toxicity study in rabbits (**RAR B. 6.2.2/03, 1992**) maternal toxicity manifested by slightly, but not statistically significantly reduced food consumption (11% reduction compared to controls on days 10-22) and bodyweight gain (19% lower than controls during the dosing period) was observed in the high dose (1000 mg/kg bw/day) animals. There were generally no differences in clinical signs between controls and the phenmedipham treated groups but two high dose (1000 mg/kg bw/day) dams were killed on days 15 and 22 because of noticeable decline in condition (off feed, weight loss). The other dam had abortion. There were no treatment related signs of general toxicity in low and intermediate dose dams. There were no clear treatment-related effects on pups (RAR). The total incidences of fetuses with variant sternebrae in mid (71 mg/kg bw/day) and high dose (1000 mg/kg/bw) groups were slightly but not statistically significantly higher than in the control group (incidences **15.5%, 29.1% and 31%** in control, mid and high dose groups, respectively). The litter incidences of this variation were essentially similar in all groups (**8/15, 8/13, 9/15 and 11/16** at 0, 5, 71 and 1000 mg/kg bw day, respectively). According to study report historical control incidences of variant sternebrae in the laboratory ranged **9.4 – 40.2%** (six studies conducted in 1991, mean no. fetuses examined 123). The in-life phase of the study (**RAR B. 6.2.2/03, 1992**) was conducted over 08-09/1991. Moreover, statistical analysis indicated that the observed differences were more affected by animal batches used than phenmedipham treatment (RAR). Therefore, slightly increased foetal incidences of variant sternebrae are not considered treatment related.

In the second rabbit developmental study (**RAR B. 6.2.2/04, 1986)** mean maternal body weight gain of the high dose group (1000 mg/kg bw/day) was slightly, not statistically significantly, reduced from gestation day 15 onwards resulting 19% decrease compared to control group over gestation days 6-19. The corrected body weight gain of the high dose group over days 0-29 was reduced by 76 % compared to control dams (RAR, Table 6.6.2-19). The total food consumption in the high dose group (1000 mg/kg bw/day) was decreased by approximately 23% compared to the control group on days 12-19 (the data was not analysed statistically). There were no significant differences between controls and treated goups in intrauterine deaths or fetal sex ratios. Mean total uterus weights and mean fetal body weights of the high dose dams were decreased by 12% and 14%, respectively, in comparison to the controls (RAR Table 6.6.2-21). The decrease in fetal body weights at high dose reached statistically significance (50.7 g ± 10.3 and 43.7 ± 4.7 in control and high dose groups, respectively).

No remarkable differences between groups in visceral abnormalities and variants were observed. One high dose fetus had cyclops with partial fusion of orbits, nasal proboscis and hydrocephaly. Unilateral ectopic kidney was observed in two fetuses in two litters in the high dose (1000 mg/kg bw/day) group and in one fetus in the mid dose group (225 mg/kg bw/day) while no cases were observed in control and low dose (50

mg/kg bw/day) groups. One fetus in the control group, however, had bilateral ectopic kidneys. Skeletal abnormalities in the form of misaligned pelvic halves with or without scoliosis were observed more frequently in phenmedipham treated groups than in the control group. However, there was no relationship between dose and number of fetuses or litters affected (Table 27). Slight misalignment of pelvic halves (no scoliosis) did show a slight increase with increasing dose in the number of fetuses and litters affected (number of fetuses/number of litters (%): **1.5/7.7, 1.5/7.7, 2.4/13.3 and 3.7/20.0** at 0, 50, 225 and 1000 mg/kg/day, respectively, Table 27). Skeletal variations in the form of variant ribs appeared to have slightly changed incidences in number of fetuses and/or litters affected in the high dose group. Skeletal data on ossification parameters showed an increase in the incidence of cranial retardation from 12% in controls to 27% in the high dose (1000 mg/kg bw day) group. The statistical significance of this difference was not analyzed. However, considering the magnitude of the effect and the fact that also fetal weights at this dose were reduced, it seems plausible that the increased incidence of cranial retardation is treatment related and secondary to maternal toxicity (RAR). Individual maternal data is not reported and therefore it is not possible to further asses whether maternal toxicity and these fetal effects (misaligned pelvic halves, variant ribs and cranial retardation) are linked.

Table 27: Selected skeletal findings in rabbit teratogenicity study (RAR B. 6.2.2/04)

	phenmedipham (mg/kg bw/day)							
	0		**50**		**225**		**1000**	
Abnormalit/Variation	No. foetuses (%)	No. litters (%)	No. foetuses (%)	No. litters (%)	No. foetuses (%)	No. litters (%)	No. foetuses (%)	No. litters (%)
Misalignment of pelvic halves; scoliosis/incipient scoliosis at the limbo-sacral border	1 (1.5)	1 (7.7)	5 (7.7)	5 (38.5)	6 (7.3)	6 (40.0)	5 (6.2)	4 (26.7)
Misalignment of pelvic halves; no scoliosis	0	0	1 (1.5)	1 (7.7)	1 (1.2)	1 (6.7)	1 (1.2)	1 (6.7)
Slight misalignment of pelvic halves; no scoliosis	1 (1.5)	1 (7.7)	1 (1.5)	1 (7.7)	2 (2.4)	2 (13.3)	3 (3.7)	3 (20.0)
Number examined (live foetuses)	68	13	65	13	82	15	81	15
Number examined (foetal deaths)	6	4	6	5	7	5	10	6
Number with major abnormalities (%)[1]	3 (4.1)	3 (23.1)	6 (8.5)	6 (46.2)	9 (10.1)	7 (46.7)	8 (8.8)	4 (26.7)

[1] One foetus may have more than one abnormality/variant

10.11.6 Comparison with the CLP criteria

According to CLP criteria substances are classified in Category 1A for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility or development in humans.

There are no epidemiological studies available on developmental effects of phenmedipham in humans. Thus, classification of phenmedipham as Repr. 1A for developmental effecs is not appropriate.

The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.

In the most reliable rat teratogenicity study on phemedipham, runts occurred in all treated groups (150, 450, 1350 mg/kg bw day, RAR B. 6.2.2/02, 1988) while no runts were reported in the concurrent control group. Runts occurred also in phenmedipham treated group (1000 mg/kg bw/day) of the preliminary limit study. The reported incidences of runts in the treated groups (no foetuses/no. litters:0.4-0.69%/4-4.8%) were remarkably higher than in the HCD from the laboratory (no. fetuses 0-0.1%). In the same study the proportion of female fetuses was statistically significantly reduced in the high dose group (1350 mg/kg bw/day) compared to concurrent controls (proportion of females 48.4% and 53.7% at high dose and controls, respectively) and ossification effects were observed that did not simply imply a general delayed ossification.

Runts, defined as small fetuses less than half of the size of their litter mates, are considered malformations and of high concern (ECETOC guidance). This finding is therefore considered the prime concern for developmental toxicity of phenmedipham. Runts occurred together with low to moderate maternal toxicity manifested by reduced body weight gains and food consumption of phenmedipham treated dams. The individual data revealed that the high dose dam which gave birth to two runts had large litter (14 fetuses) and negative corrected body weight gain, suggesting that general toxicity and large litter size could have contributed to occurrence of runts. Moreover, one small fetus (does not fulfill definition of runt) was born to a control dam which had large litter (14 fetuses) and low corrected body weight gain (Table 59). However, runts were also born to dams with small litter sizes and corrected body weight gains higher than average (low dose dam of the main study and the dam in the limit study). Hence, occurrence of runts in this study can not be considered as a secondary non-specific consequence of other toxic effects.

No runts were reported in the other rat teratogenicity study (RAR B.6.6.2/01, 1989), although higher dose range was used (0, 516, 1160 and 2580 mg/kg bw/day) and foetal body weights were significantly reduced in mid and high dose groups in this study. Likewise, runts were not reported in rat generational studies (RAR B.6.6.1/01-03). Moreover, skewed sex ratio and similar ossification effects have only been reported in this one rat teratogenicity study (RAR B. 6.2.2/02, 1988). Taken also into account that one small fetus occurred in the control group, we consider that occurrences of runts at low incidence and these other findings in one study only, do not provide clear evidence of an adverse effect on development. Therefore, classification of phenmedipham for Repr 1B for developmental toxicity may not be appropriate.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

We conclude that there is some evidence for developmental toxicity of phenmedipham and therefore classification for Category 2 is appropriate.

10.11.7 Adverse effects on or via lactation

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

In one rat two generation study slightly higher incidence of inadequate maternal care was observed in the phenmedipham treated groups compared to control group in both generations (**RAR B.6.6.1/01, 1987**). For details of the data see section 10.11.2. Increased inadequate mathernal care was associated with slightly increased early mortality of pups in the F1 generation, but similar effect on early viability of pups was not observed in F2 generation. These findings did not reach statistical significance and it remained unclear whether these effects were treatment related. Effects on maternal care or early survival of pups were not reported in other generational studies with phenmedipham (there are no information in study reports whether adequacy of maternal care was recorded). There are no data available on secretion of phenmedipham to milk.

10.11.9 Comparison with the CLP criteria

According to CLP criteria substances shall be classified for effects on or via lactation when: "substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child. This classification can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or **(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk**; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk."

There are no data available on secretion of phenmedipham to milk.

Guidance on the Application of CLP criteria (chapter 3.7.2.3.3.) further elaborates classification decision based on generational studies in animals: "The value of these studies is that they directly observe the pups during lactation and any adverse effects, such as deaths, decreased viability, clinical signs such as reduced bodyweight gain etc, can be directly observed and quantified. However, expert judgement is required to decide whether these effects in pups are due to a direct adverse effect on lactation, or are due to impaired nursing behaviour which is a non specific secondary consequence of maternal toxicity. **If the impaired nursing behaviour is proven to be a substance related specific effect on behaviour, then classification for effects on or via lactation may be appropriate**." (ECHA, 2017).

The increased incidence of inadequate maternal care in phenmedipham treated groups did not reach statistical significance and it remained unclear whether these effects were related to phenmedipham treatment. Effects on maternal care or early survival of pups were not reported in other generational studies with phenmedipham. Therefore classification for effects on or via lactation is not appropriate.

10.11.10 Conclusion on classification and labelling for reproductive toxicity

Phenmedipham is proposed to be classified Repr. 2; H361d. No classification for fertility or effects on or via lactation is proposed.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

No effects on sexual function and fertility were observed in the three available generational studies with phenmedipham, and no effects on reproductive organs were reported in repeat dose toxicity studies except a dubious finding of increased aspermatogenesis in one carcinogenicity study. The DS proposed no classification for fertility. However, they noted that EFSA considered the data on fertility inconclusive, mainly because sperm parameters, affected by desmedipham, were not investigated in studies with phenmedipham (EFSA, 2018). In addition, the DS also pointed out low dosing in two of the three generational studies.

Development

Two rat and two rabbit prenatal developmental toxicity (PNDT) studies are available, all testing up to or above the limit dose of 1 000 mg/kg bw/d. The DS proposed classification in Category 2 based on runts (small foetuses less than half of the size of their littermates) occurring at a low incidence in one of the rat PNDT studies (B.6.6.2/02). According to the DS, classification is further supported by increased

ossification and altered sex ratio in this study.

Lactation

The DS discussed a slight increase in early pup mortality associated with poor maternal care in one of the generational studies, but did not consider it to meet the criteria for classification.

Comments received during public consultation

Three MSCA and 1 Industry association provided their comments.

As to fertility, Industry and 1 MSCA supported no classification. The other two MSCAs considered the data on fertility inconclusive, one of them suggesting read-across from desmedipham.

Regarding developmental toxicity, 2 MSCAs supported Category 2 for development, while 1 MSCA questioned whether the data are sufficient for classification given the maternal toxicity, larger litter size at the high dose and occurrence of one small foetus in the control group of study B.6.6.2/02. This MSCA also pointed out the lack of developmental anomalies at higher dose levels in the other rat PNDT study (B.6.6.2/01).

The industry association proposed no classification for development, putting forward the following arguments:

- The incidence of runts did not show an obvious dose-response relationship and appeared to be associated with lower average weights of the whole litter.
- 'Runt' is not a malformation and the criteria for defining a 'runt' are subjective. Still, the ECETOC monograph 31 defines a 'runt' as a foetus, which weighs less than half of the average ligger weight. In study B.6.6.2/02, the weights of all foetuses called 'runts' were more than half of the mean foetal weight for the litter where the 'runt' was observed, when considering non-'runt' foetuses of the corresponding sex.
- Maternal corrected body weight gain was reduced by 17 % and 36 % at 450 and 1 350 mg/kg bw/d, respectively, which indicates significant maternal toxicity.
- This finding was not repeated in another rat study or in two rabbit studies that used equivalent doses.
- As to the altered sex ratio, the results did not markedly deviate from the expected percentage of 50 % for both sexes and the main reason for the statistically significant difference was the rather low percentage of males (or high percentage of females) in the control group. In addition, no such effect was observed in the pilot study or in the other rat PNDT study. This confirms that the observed changes are only due to a high variability.

The DS replied that runts occurred both in the preliminary and main study, and the individual animal data do not show a correlation between maternal toxicity and occurrence of runts. They, however, acknowledged that all pups defined as runts in the study report did not have body weights half of their littermates. Still, they were of the opinion that there is no indication that the occurrence of runts in phenmedipham-treated groups would be a secondary, non-specific consequence of maternal toxicity. As to the

sex ratios, the DS admitted that the finding might be spurious due to high variability.

One MSCA supported no classification for effects on/via lactation.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

Generational studies

Two 2-generation studies (from 1987 and 1986), generally complying with OECD TG 416 (1983), and one pre-guideline 3-generation study (from 1979) are available for phenmedipham. All studies were reportedly conducted according to the principles of GLP. RAC notes that some sensitive parameters introduced into the OECD TG 416 in 2001, such as sperm parameters or sexual maturation, were not investigated in these studies.

None of these three studies reported adverse effects related to fertility. The top doses were ca. 80, 225 and 40 mg/kg bw/d in study B.6.6.1/01, /02 and /03 respectively. The top dose selection in study B.6.6.1/02 is considered acceptable as the body weight of parental animals in this study was reduced by up to 13 % compared to controls and the maximum tolerated dose in 90-day studies appears to lie around or above 400 mg/kg bw/d (B.6.3.2/04, /05, /06). RAC agrees with the DS that parental toxicity in the other two studies (B.6.6.1/01, /03) was rather limited and higher doses should have been tested.

Repeat dose toxicity studies

According to the DS, there were no non-neoplastic histopathological findings in reproductive organs in the repeat dose toxicity studies with phenmedipham except for a slight increase in aspermatogenesis in one of the carcinogenicity studies (B.6.5.1/06), that was difficult to interpret due to poor reporting. RAC examined the study and found that the increase (9/50 vs 5/50) was not statistically significant. No such effect was seen in the other rat carcinogenicity studies testing higher doses. Thus, this finding is not relevant for classification.

Conclusion on classification for fertility and sexual function

In the absence of effects on sexual function and fertility in the available studies with phenmedipham, RAC agrees with the DS that **no classification is justified**.

Adverse effects on development

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

PNDT studies		
Type of study; Reference; Year	**Method**	**Observations**
Rat		
PNDT study, gavage B.6.6.2/01 1989	OECD TG 414 GLP Strain: Wistar Doses: 0, 516, 1 160, 2 580	Maternal toxicity All doses: • ↓ corrected bw gain (at the top dose down to 31 g vs 41 g in control GD 0-20), ↓

	mg/kg bw/d Dosing GD 6-15 22 females/group Two batches of the test substance from two different sources were used; purity of one of the batches is not known	food consumption (at the top dose by 12 % GD 6-15) <u>Developmental toxicity</u> All doses: • ↓ foetal weight (by up to 7 %) • Incomplete ossification of neck vertebrae
PNDT study, gavage B.6.6.2/02 1988	OECD TG 414 GLP Strain: Wistar/HAN Doses: 0, 150, 450, 1 350 mg/kg bw/d Dosing GD 6-15 25 females/group	<u>Maternal toxicity</u> 1 350 mg/kg bw/d: • ↓ corrected bw gain (4.2 % vs 6.6 % in control), ↓ food consumption (by 7 % GD 6-16); corrected bw reduced by 3 % (not stat. sign.) 450 mg/kg bw/d: • ↓ corrected bw gain (5.5 % vs 6.6 % in control) 150 mg/kg bw/d: no adverse effects <u>Developmental toxicity</u> 1 350 mg/kg bw/d: • 2 'runts' in 1 litter • ↓ incidence of non-ossified metatarsalia 450 mg/kg bw/d: • 1 'runt' 150 mg/kg bw/d: • 1 'runt'
Rabbit		
PNDT study, gavage B.6.6.2/03 1992	OECD TG 414 GLP Strain: New Zealand White Doses: 0, 5, 71, 1 000 mg/kg bw/d Dosing GD 6-18 16-21 females/group	<u>Maternal toxicity</u> 1 000 mg/kg bw/d: • 2 out of 21 animals sacrificed due to poor condition • ↓ food consumption (by 8 % GD 6-18), ↓ bw gain 71 mg/kg bw/d: no adverse effects <u>Developmental toxicity</u> ≤ 1 000 mg/kg bw/d: no adverse effects
PNDT study, gavage B.6.6.2/04 1986	OECD TG 414 GLP Strain: New Zealand White Doses: 0, 50, 225, 1 000 mg/kg bw/d Dosing GD 6-18	<u>Maternal toxicity</u> 1 000 mg/kg bw/d: • ↓ food consumption (by 13 % GD 6-18), ↓ bw gain ≤ 225 mg/kg bw/d: no adverse effects

	15 females/group	Developmental toxicity 1 000 mg/kg bw/d: • ↓ foetal weight (by 14 %) • Reduced ossification (cranium) • Slight increase in misaligned pelvic halves 225 mg/kg bw/d: • Slight increase in misaligned pelvic halves 50 mg/kg bw/d: • Slight increase in misaligned pelvic halves

GD=gestation day

Rat PNDT study B.6.6.2/01

No effects warranting classification were observed in this study up to the very high top dose of 2 580 mg/kg bw/d. RAC notes that two different batches of the test substance from two different sources were used, and that purity of one of the batches is unknown.

Rat PNDT study B.6.6.2/02

The only finding potentially warranting classification is the occurrence of abnormally small foetuses ('runts'). The slightly changed sex ratio at the top dose (males 52 % vs 46 % in the control) reflects normal variability and the slightly reduced incidence of non-ossified metatarsalia is not an effect warranting classification.

Maternal toxicity was rather limited even at the top dose of 1 350 mg/kg bw/d. The data on the abnormally small foetuses are provided in the table below. Their weight was from 2.2 to 2.6 g (53-68 % of the average weight of their littermates of the same sex). For comparison, the lowest individual foetal weight in the control group was 3.3 g (no abnormalities on external and visceral examination; dam no. 16). Both 'runts' examined for skeletal anomalies showed retarded ossification and the mid-dose 'runt' additionally several malformations (ribs missing, ribs fused, fused vertebral centra). HCD from 2 years (1985-86) preceding the current study (1987) reported 3 'runts' among ca. 3 700 control foetuses.

Data on abnormally small foetuses in the rat PNDT study B.6.6.2/02

Dose (mg/kg bw/d)	**0**	**150**	**450**	**1 350**
Number of foetuses (litters) examined	293 (24)	250 (23)	241 (21)	289 (25)
Runt: foetal (litter) incidence	0	1 (1)	1 (1)	2 (1)
Runt weight [g], sex	–	2.5 ♂	2.2 ♀	2.6 ♀; 2.4 ♀
Findings on visceral and skeletal examination of the runt	–	None	Retarded ossification and several anomalies	'runt' 1: none 'runt' 2: retarded ossification
Mean foetal weight for the "runt litter", only foetuses of the corresponding sex, runts	–	4.7	4.1	3.8

excluded [g]				
Range of foetal weights in the "runt litter", both sexes, runts excluded	–	4.2-4.8	3.6-4.8	3.7-4.5
Group mean foetal weight, combined sexes [g]; ±SD	4.6 (±0.3)	4.6 (±0.4)	4.7 (±0.4)	4.6 (±0.4)
Size of the affected litter	–	7	12	14
Group mean litter size	12.2	10.9	11.5	11.6
Corrected bw gain (GD 6-21) of the affected dam [% of weight on GD 6]	–	14.9	9.3	–0.4
Group mean corrected bw gain (GD 6-21) [% of weight on GD 6]; ±SD	6.6 (±3.7)	6.9 (±4.6)	5.5 (±6.1)	4.2 (±3.9)

Two small foetuses (2.6 g and 3.0 g) in one out of five litters were also observed at 1 000 mg/kg bw/d in the preliminary study. However, both these foetuses were malformed (hydrocephaly, one also had brachygnathia) and as malformed foetuses usually have lower weight, it is not clear whether this finding corresponds to 'runts' in the main study.

In summary, there were single incidences of abnormally small foetuses at the low- and mid-dose in the absence of maternal toxicity. The top dose of 1 350 mg/kg bw/d exceeds the limit dose for an OECD TG 414 study of 1 000 mg/kg bw/d, so the findings at this dose are considered less relevant for classification. Still, the two small foetuses at the top dose indicate that the findings at the low and mid-dose are treatment-related. The dose-response curve is rather shallow.

Rabbit PNDT studies B.6.6.2/03 and B.6.6.2/04

Study B.6.6.2/03 was negative regarding developmental toxicity, while study B.6.6.2/04 showed several relatively minor effects: reduced foetal weight, delayed ossification and slightly increased incidence of misaligned pelvic halves.

The foetal weight reduction by 14 % occurred in presence of some maternal toxicity, and is not considered to be of sufficient magnitude to warrant classification. The delayed ossification of the cranium is likely to reflect a slight general developmental delay. The incidences of pelvic anomalies are presented in the following table. The concern about these anomalies is reduced by their presence in the control group, lack of a dose-response relationship for misalignment with scoliosis, and lack of this effect in the other rabbit study. Therefore, the occurrence of misaligned pelvic halves in the treated groups is not considered to contribute to classification.

Pelvic anomalies in the rabbit PNDT study B.6.6.2/04				
Dose (mg/kg bw/d)	**0**	**50**	**225**	**1 000**
Number of foetuses (litters) examined	68 (13)	65 (13)	82 (15)	81 (15)
Misalignment of pelvic halves; scoliosis/incipient scoliosis at the	1 (1)	5 (5)	6 (6)	5 (4)

lumbo-sacral border; foetuses (litters)				
Misalignment of pelvic halves; no scoliosis	0	1 (1)	1 (1)	1 (1)
Slight misalignment of pelvic halves; no scoliosis	1 (1)	1 (1)	2 (2)	3 (3)

Conclusion on classification for developmental toxicity

The only finding to be considered for classification is the occurrence of abnormally small foetuses (weighing approx. half that of their litter mates) at doses without maternal toxicity in the rat PNDT study (B.6.6.2/02). The corresponding finding in humans, intrauterine growth restriction, is associated with increased risk of neonatal mortality and neurodevelopmental problems. Thus, although the finding is not a malformation (it does not necessarily lead to permanent damage), the level of concern is higher than with a variation.

On the other hand, the concern is reduced by the low incidence (only single incidences per group below the limit dose), very shallow dose-response curve (no increase in litter incidence from 150 to 1 350 mg/kg bw/d) and lack of such effects in the other rat PNDT study (B.6.6.2/01) testing up to 2 580 mg/kg bw/d.

Taking into account the very low incidence of abnormally small foetuses, the very shallow dose-response curve and inconsistent results between studies, RAC is of the opinion that **classification for developmental toxicity is not warranted**.

Adverse effects on or via lactation

The 2-generation study (B.6.6.1/01) reported an increase in early pup mortality in the P/F1 generation correlating with deficient maternal care. There was no such effect in the second generation, or in the other 2-generation study (B.6.6.1/02) testing higher doses.

The 2-generation study (B.6.6.1/02) reported a reduction in F2 pup body weights. The birth weight was unaffected, but from PND 14 the pup weight started to differ significantly from controls at the top dose of 225 mg/kg bw/d (by 15 % on PND 14, by 18 % on PND 21). However, by that time the pups already start feeding on the maternal diet, so the effect cannot be unequivocally attributed to lactation. In addition, the dose was maternally toxic as indicated by a maternal body weight reduction by 13 % compared to controls, which might have adversely affected milk production or maternal care as a non-specific secondary effect.

Conclusion on classification for lactation

The slightly increased pup mortality in study B.6.6.1/01 was associated with poor maternal care and was not seen in study B.6.6.1/02 at a higher dose. The pup weight reduction in study B.6.6.1/02 was not observed before PND 14 and was associated with maternal toxicity. Therefore, RAC agrees with the DS that **no classification for effects on or via lactation is warranted**.

Overall conclusion on reproductive toxicity

RAC is of the opinion that **classification of phenmedipham for reproductive toxicity is not warranted.**

10.12 Specific target organ toxicity-single exposure

10.13 Specific target organ toxicity-repeated exposure

Table 28 Summary table of short-term animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**		**Reference**
		NOAEL	**LOAEL**	
Rats				
13-week oral toxicity study in rats OECD test guideline 408, GLP rat, Sprague-Dawley 10/sex/group	Phenmedipham (purity: 98.5%) oral, diet 0, 400, 800, 1200 ppm M: 30, 60 and 92 mg/kg bw/day F: 33, 72 and 122 mg/kg bw/day exposure: 13 weeks	<400 ppm M: <30 mg/kg bw/day F: <33 mg/kg bw/day	400 ppm Mild decreases of red blood cell parameters (Hb, Hct, RBC), hemosiderin deposition (spleen, liver, kidneys), extramedullary hematopoiesis in the spleen, organ weight changes (spleen ↑ in males, uterus and thymus ↓ in females)	1986a RAR B.6.3.2/02 M-146355-02-1
13-week oral toxicity study with 4-week recovery period in rats OECD test guideline 408, GLP rat, Sprague-Dawley 10/sex/group	Phenmedipham (purity: 97% ± 1% concentration, impurities: max 1.5% Methyl-N-(3-hydroxyphenyl) carbonate (MHPC) and max. 1.5% N,N'-Di-m-tolyurea (DTU)) oral, diet 0, 150, 500, 1500 ppm M: 13, 43 and 131 mg/ kg bw/day F: 16, 51 and 149 mg/ kg bw/day exposure: 13 weeks	150 ppm M: 13 mg/kg bw/day F: 16 mg/kg bw/day	500 ppm Hemosiderin deposition (spleen, liver, kidneys), Supported by minimal anemia (decreased Hb, Hct, RBC), decreased pituitary weight (males), WBC increase (females).	1986b RAR B.6.3.2/01 M-146380-03-1

13-week oral toxicity study in rats OECD test guideline 408, GLP (formulated product and one dose group with active substance) rat, Sprague-Dawley 15/sex/group	The purity of the active substance is unknown the content of impurities/co-formulants in the formulated product is unknown. oral, diet 40, 400, 4000 ppm formulated product, 600 ppm phenmedipham M: 0.5, 5 and 41 (FP) and 44 (AI) mg/kg bw/day F: 0.5, 5 and 46 (FP) and 48 (AI) mg/kg bw/day exposure: 13 weeks	Supplemental study The study was not used for conclusions about the active substance.	- Effects at 600 ppm PMP: Decreased red blood cell parameters (Hb, Hct, RBC) in females and decreased platelets in males, organ weight changes in males (pituitary ↓, kidneys ↓, spleen ↑)	1982 RAR B.6.3.2/03 M-146373-01-2
13-week oral toxicity study in rats OECD test guideline 408, GLP rat, Fischer 344 20/sex/group	Phenmedipham (purity: 98.6%) oral, diet 0, 50, 500, 5000 ppm M: 3.5, 35, 367 mg/kg bw/day F: 3.7, 37, 378 mg/kg bw/day exposure: 13 weeks	50 ppm M: 3.5 mg/kg bw F: 3.7 mg/kg bw/day	500 ppm Hemosiderin deposition (spleen), enlarged and/or black spleen, organ weight changes (spleen ↑, adrenals ↑), minimal anemia (reduced Hb, Hct, RBC; increased number of reticulocytes), changes in WBC (lymphocytes, neu-trophils).	1981 RAR B.6.3.2/04 M-145614-01-1
90-day oral toxicity study in the rat OECD test guideline 408, GLP rat, Sprague-Dawley 10/sex/group	phenmedipham technical (purity 98.4%) 0, 1000, 5000 and 20000 ppm oral, diet M: 75, 389, 1555 mg/ kg bw/day F: 85, 434, 1723 mg/kg bw/day exposure: 90 days	<1000 ppm M: <75 mg/kg bw F: <85 mg/kg bw/day	1000 ppm Hemosiderin deposition (male liver and kidneys), MetHb ↑, MCV ↑, relative testes weight ↑.	1987 RAR B.6.3.2/05 M-502068-01-1

90-day oral toxicity study in the rat OECD test guideline 408 (1998) GLP Only minor deviations. not considered to affect the results of the study rat, Wistar 10/sex/group	Phenmedipharm (purity: 98.4 %) oral, diet 0, 1000, 3000, 10000 and 20000 ppm M: 61, 189, 636, 1 239 mg/kg bw/day F: 71, 214, 658, 1309 mg/kg bw/day exposure: 90 days	<1000 ppm M: <61 mg/kg bw/day F: <71 mg/kg bw/day	1000 ppm Reduced Hb, Hct and RBC, increased MetHb, Heinz bodies and reticulocytes, hemosiderin deposition and extramedullary haematopoiesis (liver and spleen), organ weight changes (spleen (M)↑, prostate↓, pituitary (F) ↓, adrenal diffuse vacuolation (M)	2002 RAR B.6.3.2/06 M-211096-01-1
Mice				
8-week oral study in the mouse GLP compliance (OECD 1982) and according to OECD test guideline 407 mouse, Swiss CD-1 10/sex/group	Phenmedipham (purity 99.3%) oral, diet 0, 1000, 5000, 15000 ppm M: 125, 623 and 1927 mg/kg bw/day F: 144, 699 and 2071 mg/kg bw/day exposure: 8 weeks	1000 ppm M: 125 mg/kg bw/day F: 144 mg/kg bw/	5000 ppm Methaemoglobinemia, increased liver weights (males), brown pigment in hepatic Kupffer cells, reduced Hb, Hct, RBC.	1985 RAR B.6.3.1/01 M-145645-01-1
Dogs				
60-day oral toxicity study in the dog OECD test guideline 409 (1998) GLP (GLP compliance cannot be claimed for the experimental findings reported) Beagle dog 4/sex/group	Phenmedipham (purity: 98.4%) oral, diet 0, 300, 3000 and 30000 ppm M: 11.3, 118, 1199 mg/kg bw/day F: 11.8, 123, 1086 mg/kg bw/day exposure: 60 days	<300 ppm M: <11.3 mg/ kg/day F: <11.8 mg/kg/day	300 ppm Reduced BW gain in males, organ weight changes (thyroid ↑, testes↓ ovaries↑), possibly thyroid hypertrophy.	2001 RAR B.6.3.1/02 M-199382-02-1
104-week oral toxicity study in dogs No test guideline, no GLP Beagle dog 8/sex/group	Phenmedipham (technical, claimed purity 100%) oral, diet 0, 40, 200 and 1000 ppm M: 1, 6, 28 mg/kg bw/ day F: 1, 6, 27 mg/kg bw/ day exposure: 104 weeks	>1000 ppm M: >28 mg/kg bw/day F: >27 mg/kg bw/day	> 1000 ppm No treatment-related changes observed	1980 RAR B.6.3.2/08 M-145590-01-1

18-week repeated feeding study in dogs No test guideline, no GLP single group of Beagle dogs (2 males and females)	Phenmedipham technical ("pure" active ingredient, ZK 15320) oral, gelatine capsules One group receiving successively higher doses (250, 500 and 1000 mg/kg bw/day) of phenmedipham exposure: 18 weeks	No NOAEL can be derived due to the unusual treatment conditions	No LOAEL can be derived because of the unusual treatment conditions Treatment-related findings: Hct ↓, RBC ↓, hypochromic erythrocytes, anisocytosis, spleen focal hematopoiesis and follicular hyperplasia	1968 RAR B.6.3.2/07 M-145585-01-2

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

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

Ten short-term toxicity studies are available on phenedipham (Table 28). Chronic toxicity/carcinogenicity studies and reproductive toxicity studies are reported in the sections 10.9 and 10.10. Further details are given in RAR.

Repeated dose toxicity on phenmedipham have been conducted in mice, rats and dogs using oral administration. The majority of the short-term toxicity studies are rather old and are not fully in compliance with the current OECD test guidelines. The studies were only done with oral administration, in most cases by mixing the test substance in feed, in rat, mouse and dog. No studies were performed by dermal application of or inhalatory exposure to phenmedipham.

Rats

Subacute and subchronic effects of phenmedipham in rats were examined in six short-term toxicity studies.

Formulated product along with pure phenmedipham was used in one of the rat studies (1982, RAR B.6.3.2/03). The content of impurities/co-formulants in the formulated product is unknown. The concentration of the" pure" active substance in the study (one dose only) is not given, as was the case also with the test substances in the dog study (1968, RAR B.6.3.2/07). The test substance purity in the study RAR B.6.3.2/08(1980) was claimed to be 100%. In the study RAR B.6.3.1/02 (2001), the purity of the technical material was not given in the study report and is therefore assumed to be 98.4% w/w, which is the purity given in another study using the same batch number.

The most consistent observations related to treatment with phenmedipham in rats were hematological effects, mainly on red blood cell parameters. These effects included slight methemoglobinemia and reduced RBC counts, Hb, PCV and increased MCH. The highest reduction in hemoglobin (Hb) level in blood was up to 18 % in comparison with controls at 658 mg/kg bw/day (10000 ppm) in RAR B.6.3.2/06 (2002) study. The highest observed methemoglobin values were 2.8 – 3.2 % in rat at approximately 1500 mg/kg bw/day (0.6 – 0.8 % in controls) (1987, RAR B.6.3.2/05); in five of the studies, methemoglobin levels were not determined. Hemosiderin deposition was generally observed in the liver and in the spleen, but in some cases also in the kidneys in rats. Increased liver and spleen weights were also noted, and in some cases also kidney weight changes were noted. Occasionally, increased bilirubin counts were observed. Increased hematopoiesis in the bone marrow and increased reticulocyte counts in blood were also observed. The major findings in rat are consistent with effects pointing towards minimal to moderate methemoglobinemia, leading to slight to moderate changes in RBC parameters and hemolytic anemia, increased activities of the liver and spleen - the organs mainly involved in the turnover of red blood cells - and compensatory medullary hematopoiesis. Extramedullary hematopoiesis was also observed (in liver and spleen).

In addition to weight changes in liver, spleen and kidneys, occasional weight changes were also observed in endocrine organs (decreases in pituitary, uterus, prostate and thymus, increases in testes and adrenals).

Increased cholinesterase activities in plasma, red blood cells and brain were also noted in practically all rat studies. The biological significance of this observation is unclear. The increased plasma cholinesterase activity may be due to cholinesterase released from the hemolyzed red blood cells.

One study in rats (1986b, RAR B.6.3.2/01) showed that the changes in RBC parameters induced by a 13-week feeding study were almost completely reversible during a 4 week recovery period (2 – 4% increases still observed), as were the increases in hemosiderin deposition in liver and kidneys. In spleen, hemosiderin deposition was still marked and females had reduced body weights. The overall NOAEL in rats was 3.5 mg/kg bw/day (1981, RAR B.6.3.2/04).

Mice

The 8 week study in mice with oral administration (1985, RAR B.6.3.1/01) gave the same type of effects as seen in the rat studies. Again slight anemia was noted together with increased liver weight, and hemosiderin deposition in the liver. In addition to this also increased methemoglobin levels were observed (4.0 – 4.5% at approximately 2000 mg/kg bw/day compared to 2.3% in controls), and an increase in hepatic extramedullary hematopoiesis. The reduction in hemoglobin (Hb) level in blood was up to 18 % at approximately 2000 mg/kg bw/day(15000 ppm) in comparison with controls. The NOAEL in mice is 125 mg/kg bw/day.

Dogs

Hematotoxic effects were also observed in dogs. In the 60-day study in dogs (2001, RAR B.6.3.1/02), signs of slight anemia included increased methemoglobin values (4.2 – 5.1% at approximately 1150 mg/kg bw/day compared to 0.5-0.6% in controls), increased number of reticulocytes, bone marrow effects and extramedullary hematopoiesis. The reduction in hemoglobin (Hb) level in blood was up to 27 % at approximately 1200 mg/kg bw/day (30000 ppm) in comparison with controls. At the LOAEL, absolute and relative thyroid weight and the absolute weight of ovaries were increased while the body weight gain and the absolute testes weight were reduced in males. Due to a rather unusual treatment regime in the dog study RAR B.6.3.2/07(1968), no conclusions between the applied doses and observed effects could be made. It may however be noted that decreases in red blood cell parameters were seen together with extramedullary hematopoiesis, again indicating slight anemia. Transient hematological changes were also observed in the 2-year dog study (1980, RAR B.6.3.2/08). The overall NOAEL in dogs is lower than 11.3 mg/kg bw/day.

The commonly observed effect of repeated exposure to phenmedipham is a slight (up to 3.2 % in rat and 5% measured in dogs) increase in methemoglobin levels. It should be noted that methemoglobin was measured in only four of the short-term studies and the earliest measures are after 4 weeks of treatment. Mostly moderate and reversible (within 4 weeks in rats after cessation of dosing) hematological changes in red blood cell parameters, liver and spleen weight changes (sometimes also in kidneys), hemosiderin deposition in spleen, liver and kidneys, and increased medullary and/or extramedullary hematopoiesis is seen as a secondary reaction to the increased methemoglobin levels.

An overall NOAEL for the short-term toxicity studies is based on the 90-day rat study RAR B.6.3.2/04 (1981) with the lowest NOAEL of 3.5 mg/kg bw/day despite a ten-fold dose spacing used in the study. This overall NOAEL is supported by the 60-day dog study in which a NOAEL could not be established but could be estimated from a LOAEL of 11.3 mg/kg bw/day to an estimated NOAEL of 3.8 mg/kg bw/day by using an uncertainty factor of 3.

10.13.2 Comparison with the CLP criteria

As described above, target organ of toxicity in experimental animals following repeated oral administration of phenmedipham is the blood (haematopoietic system). However, the relevancy of these findings has to be weighted and compared with the CLP classification criteria.

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

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

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

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

Haematological effects

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

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

The guidance on the application of the CLP criteria also states that in the case where multiple less severe effects with regenerative capacity were observed, the classification should apply as "Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs." (CLP Annex I, 3.9.1.4).

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

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

The following tables (Table 29 and Table 30) summarises the key haematological findings from relevant studies as they relate to the guidance values for classification. The information on effects after short-term repeated exposure was complemented by the non-neoplastic results from the combined chronic toxicity and carcinogenicity studies in mice and rats. In addition, the maternal toxicity data of the oral developmental toxicity studies in rats and rabbits and of a two-generation toxicity studies in rats were considered for the evaluation of the specific target organ toxicity after repeated exposure of phenmedipham.

Table 29 Summary table of haematological findings in relevant repeated dose toxicity studies

Study	**Doses relevant for STOT RE**	**Effects at this dose level**
Rats		
13-week oral toxicity study in the rats OECD test guideline 408, GLP rat, Sprague-Dawley 10/sex/group, oral (diet) 0, 400, 800, 1200 ppm M: 30, 60 and 92 mg/kg bw/day F: 33, 72 and 122 mg/kg bw/day Blood samples for haematological measurements were collected during weeks 6 and 13 1986a RAR B.6.3.2/02 M-146355-02-1	*Cat 2: 10 < C ≤ 100 mg/kg bw/day*	at 400 ppm - ↓ Hb in males (3 %* at week 6) and females (7 %*** at week 6 and 13) - ↓ RBC in females (8 %*** at week 6 and 13) - ↓ PCV in males (3%* at week 6) and females (5 %*** at week 6 and 7 %*** at week 13) - ↑ hemosiderin deposition in the spleen (very mild to moderate) and in liver in males and females. Kidneys in males (very mild to moderate) - ↑ spleen weight in males (relative 21 %**) at 800 ppm - ↓ Hb and RBC in males (4%** and 6 %** at week 6) - ↓ Hb (7 %*** at week 6 and 5%** at week 13) and RBC (8%*** at week 6 and 13) in females - ↓ PCV in males (5 %** at week 6 and 2.5 %* at week 13) and in females (5 %*** at week 6 and 7 %*** at week 13) - ↑ hemosiderin deposition spleen (very mild to moderate), liver (5*/10 and 8**/10 vs no incidence in controls) and kidneys (very mild to severe) in males and females - ↑ extramedullary hematopoiesis in the spleen - ↑ spleen weight in males (relative 14 %*)

		at 1200 ppm - ↓ Hb (5 %*** at week 6 and 4 %* at week 13) and RBC (9 %*** at week 6 and 7 %* at week 13) in males - ↓ Hb (8 %*** at week 6 and 13) and RBC (9 %*** at week 6 and 10 %*** at week 13) in females - ↓ PCV in males (5 %*** at week 6 and 5 %* at week 13) and in females (8 %*** at week 6 and 10 %*** at week 13) - ↑ hemosiderin deposition in the liver in males and females (8**/10 and 4/10 vs no incidence in controls). Severity not reported (amount small). - ↑ hemosiderin deposition (very mild to severe) in the spleen in males and females (males: 4/10 moderate and 1/10 severe vs 1/10 moderate in controls; females: 2 /10 moderate vs no incidences of this severity in controls) - ↑ hemosiderin deposition (very mild to severe) in the kidney in males and females (males: 2/10 moderate and 1/10 severe vs 1/10 moderate in controls; females: 4/10 moderate vs no incidences of this severity in controls) - ↑ extramedullary haematopoiesis in the spleen - ↑ spleen weight in males (relative 27 %**) - ↑ liver weight in females (relative 9 %**)
13-week oral toxicity study with 4-week recovery period in rats OECD test guideline 408, GLP rat, Sprague-Dawley 10/sex/group 0, 150, 500, 1500 ppm M: 13, 43 and 131 mg/ kg bw/day F: 16, 51 and 149 mg/ kg bw/day Blood samples for haematological measurements were collected during weeks 6, 13 and 17 1986b RAR B.6.3.2/01 M-146380-03-1	Cat 2: 10 < C ≤ 100 mg/kg bw/day	at 500 ppm - ↓ Hb in males and females (up to 4 %*), PCV (7 %**) in males, and RBC (6 %*) in females - ↑ Hemosiderin deposition in the liver (equivocal to slight) and kidneys (equivocal to severe) in males (8**/10 vs 0/10 in controls and 9*/10 vs 3/10 in controls) - ↑ severity of hemosiderin deposition in the spleen in females (5*/10 marked vs no incidences of this severity in controls) - All changes were reversible after the recovery period
13-week oral toxicity study in rats OECD test guideline 408, GLP (formulated product and one dose group with active substance) rat, Sprague-Dawley	Cat 2: 10 < C ≤ 100 mg/kg bw/day	at 600 ppm: - ↓ red blood cell parameters (Hb, PCV, RBC) in females and decreased platelets in males,↓ kidney weight and ↑spleen weight in males

15/sex/group, oral (diet) 40, 400, 4000 ppm formulated product, 600 ppm phenmedipham M: 0.5, 5 and 41 (FP) and 44 (AI) mg/kg bw/day F: 0.5, 5 and 46 (FP) and 48 (AI) mg/kg bw/day Blood samples for haematological measurements were collected during weeks 4 and 12 1982 RAR B.6.3.2/03 M-146373-01-2		
13-week oral toxicity study in rats OECD test guideline 408, GLP rat, Fischer 344 20/sex/group, oral (diet) 0, 50, 500, 5000 ppm M: 3.5, 35, 367 mg/kg bw/day F: 3.7, 37, 378 mg/kg bw/day Haematological parameters were measured after 7 and 13 weeks of treatment 1981 RAR B.6.3.2/04 M-145614-01-1	*Cat 1: C ≤ 10 mg/kg bw/day* *Cat 2: 10 < C ≤ 100 mg/kg bw/day*	at 500 ppm - ↓ Hb (up to 4%**), Hct (up to 7 %***), RBC (up to 8 %***) in males and females, ↑ reticulocytes in males and females - ↑ severity of hemosiderin deposition in the spleen in females (13/20 slight and 7/20 moderate vs 18/20 slight and 2/20 moderate in controls) - ↑ spleen weight in both sexes (absolute: 8 %* in males and 8 %** in females, relative 5 %* in males and 8 %*** in females) and ↓ kidneys weight (absolute and relative 4 %* in females and relative 4%* in males) - Enlarged and/or black spleen in 4 males and 6 females
90-day oral toxicity study in the rat OECD test guideline 408, GLP rat, Sprague-Dawley 10/sex/group, oral (diet) 0, 1000, 5000 and 20000 ppm M: 75, 389, 1555 mg/ kg bw/day F: 85, 434, 1723 mg/kg bw/day Haematological parameters were measured during week 12 1987 RAR B.6.3.2/05 M-502068-01-1	*Cat 2: 10 < C ≤ 100 mg/kg bw/day*	at 1000 ppm - ↓ Hb (8 %* in males and 9 % in females) and RBC (7 %* in males and 13 % in females) - ↑ MetHb 57 %* in females and 105 % in males compared to controls (% of Hb: 1.30* vs 0.83 and 1.19 vs 0.58) - ↑ hemosiderin deposition in the liver (6/10 vs no incidence in controls, minimal to slight) and kidneys (5/10 vs no incidence in controls, minimal to moderate) in males
90-day oral toxicity study in the rat	*After 4 weeks Cat 2: C ≤ 300*	at 1000 ppm - ↓ Hb (up to 8 %*** in females and 6 %*** in males),

OECD test guideline 408 (1998), GLP rat, Wistar 10/sex/group, oral (diet) 0, 1000, 3000, 10000 and 20000 ppm M: 61, 189, 636, 1 239 mg/kg bw/day F: 71, 214, 658, 1309 mg/kg bw/day Blood samples for haematological measurements were collected during weeks 4, 8 and 12 2002 RAR B.6.3.2/06 M-211096-01-1	*mg/kg bw/day* *After 12 weeks Cat 2: 10 < C ≤ 100 mg/kg bw/day*	Hct (up to 10 %*** in females and 8 %*** in males) and RBC (up to 9 %** in males and 8 % *** in females) - ↑ MetHb 125 %* in males at week 4 and 8 (% of Hb: up to 0.9* vs 0.4 in controls) but ↓ at week 12.↑ MetHb 9 % in females at week 12 (% of Hb: 1.08 vs 0.99 in controls) but ↓ at week 4 and 8. - ↑ Heinz bodies 350 %** in males and 673 %*** in females (up to 0.9** vs 0.2 % in controls and up to 0.85*** vs 0.11 % in controls) - ↑ reticulocytes in males and females - ↑ hemosiderin deposition (slight) in the liver 4/9 in males and 5/10 in females vs no incidence in controls - ↑ extramedullary haematopoiesis in the liver in males (5/9 vs 2/10 in controls) - ↑ hemosiderin deposition in the spleen in females (9/10 mild and 1/10 moderate vs 5/10 slight and 5/10 mild in controls) - ↑ extramedullary haematopoiesis in the spleen in females (1/10 slight, 8/10 mild and 1/10 moderate vs 5/10 slight and 5/10 mild in controls) - anisochromia and anicytosis in erythrocytes in both sex. Macrocytosis of erythrocytes in males - ↑ liver weight in females (relative 8 %*) - ↑ spleen weight (absolute and relative 13 % in males and absolute 11 % in females) at 3000 ppm (week 4) - ↓ Hb (17 %*** in females and 3 % in males), Hct (18 % *** in females and 6 % ** in males) and RBC (18 %*** in females and 4 % in males) - ↑ MetHb 175 %* in males at week 4 (% of Hb: up to 1.1 vs 0.4) but ↓ at week 12. ↑ MetHb 32 % in females (up to 1.1 vs 0.8) but ↓ at week 8 and 12 - ↑ Heinz bodies 1100 %*** in females and 600 %*** in males (1.4 %*** vs 0.1 % in controls and 1.4*** % vs 0.2 % in controls) - ↑ reticulocytes in males and females
Mice		
8-week oral study in the mouse GLP compliance (OECD 1982) and according to OECD test guideline 407 mouse, Swiss CD-1 10/sex/group, oral (diet) 0, 1000, 5000, 15000 ppm M: 125, 623 and 1927 mg/kg bw/day	*Cat 2: 16 < C ≤ 160 mg/kg bw/day*	at 1000 ppm - ↓ Hb (5 %*), RBC (9 %*) and PCV (8 %**) in males - ↑ MetHb 12 % in males and 10.9 % in females (% of Hb: 2.52 vs 2.26 and 2.54 vs 2.29) - ↓ spleen weight in males (relative 37 %**) and females (39 %)

F: 144, 699 and 2071 mg/kg bw/day Haematological measurements were conducted prior to treatment and during week 8 1985 RAR B.6.3.1/01 M-145645-01-1		
Dogs		
60-day oral toxicity study in the dog OECD test guideline 409 (1998) GLP (GLP compliance cannot be claimed for the experimental findings reported) Beagle dog 4/sex/group, oral (diet) 0, 300, 3000 and 30000 ppm M: 11.3, 118, 1199 mg/kg bw/day F: 11.8, 123, 1086 mg/kg bw/day Blood samples for haematological measurements were collected prior to treatment and during weeks 4 and 9 (additional samples in week 6) of treatment 2001 RAR B.6.3.1/02 M-199382-02-1	Cat 1: ≤ 15 mg/kg bw/day Cat 2: 15 < C ≤ 150 mg/kg bw/day	at 300 ppm - ↑ MetHb 100 % in males after 9 weeks (% of Hb: 1.0 vs 0.5 in controls) - ↑ reticulocytes in both sex at week 4 (38-67 %) and females also at week 9 (26 %) - ↓ myeloid: erythroid ratio in both sexes in the bone marrow (10-16 %) - extramedullary hematopoiesis (minimal) in the spleen in 1/4 males at 3000 ppm - ↓ Hb (4 %), RBC (7 %) and Hct (3 %) in males after 9 weeks - ↑ reticulocytes 133 %** in males and 62 % in females after 4 weeks and 19 -31 % at week 9 - ↑ MetHb 480 %** in males and 300 %*** in females at week 9 (% of Hb: 2.9** vs 0.5 and 2.4*** vs 0.6 in controls) - ↑ Heinz bodies in males (1.1 % vs 0.5 % in controls) - ↑ liver weight in females (absolute 19 % and relative 18 %) - ↑ spleen weight (absolute in males 17 % and females 22 %) in both sexes and relative in females (50 %) - ↑ cellularity in the bone marrow in females (3/4 moderate and 1/4 severe vs 2/4 moderate in controls) - ↓ myeloid: erythroid ratio in both sexes in the bone marrow (25-33 %) - ↑ hemosiderin deposition (slight) in the spleen in 1/4 males - ↑ extramedullary hematopoiesis in the spleen in 1/4 males (slight) and 1/4 in females (minimal) vs no incidence in controls

* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

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

Table 30 Summary table of haematological findings in relevant long-term toxicity/carcinogenicity studies

Study	Doses relevant for STOT RE	Effects at this dose level
12-month oral toxicity study in rats OECD test guideline 452/453 (1981), GLP Rat, Sprague-Dawley 20/sex/group, oral (diet) 0, 60, 250, 1000 ppm M: 3, 15, 59 mg/kg bw/day F: 5, 19, 78 mg/kg bw/day 1988a B.6.5.1./04 M-146365-02-1 2000 RAR B.6.5.1/01 M-199354-01-1	*Cat 1: C ≤ 2.5 mg/kg bw/day* *Cat 2: 2.5 < C ≤ 25 mg/kg bw/day*	at 250 ppm - ↓ Hb in males (5 %*) and in females (3 %) - ↓ Hct in males (6 %***) and in females (4 %*), - ↑ haemosiderin deposition in the liver in females - blood pigment in urine
12-month oral toxicity study in rats OECD test guideline 452/453 (1981), GLP Rat, Sprague-Dawley 20/sex/group, oral (diet) 0, 60, 250, 1000 ppm M: 4, 17, 70 mg/kg bw/day F: 5, 20, 84 mg/kg bw/day 1987 RAR B.6.5.1/02 M-146386-03-1	*Cat 2: 2.5 < C ≤ 25 mg/kg bw/day*	at 250 ppm - ↓ Hb (4 %*), RBC (3-6 %*), Hct (4 %*) in males and females - ↑ hemosiderin deposition in the kidneys in male
24-month oral carcinogenicity study in rats OECD test guideline 453, GLP Rat, Sprague-Dawley 50/sex/group, oral (diet) 0, 60, 250, 1000 ppm M: 3, 13, 50 mg/kg bw/day F: 4, 17, 68 mg/kg bw/day 1988b RAR B.6.5.1/03	*Cat 2: 1.25 < C ≤ 12.5 mg/kg bw/day*	at 250 ppm - ↑ hemosiderin deposition in the liver and spleen

M-146366-02-1 2000 RAR B.6.5.1/04 M-199354-01-1		
24-month oral carcinogenicity study in rats OECD test guideline 453, GLP Rat, Sprague-Dawley 50/sex/group, oral (diet) 0, 60, 250,1000 ppm M: 3, 14, 55 mg/kg bw/ day F: 4, 18, 73 mg/kg bw/ day 1988c RAR B.6.5.1/05 M-146387-03-1	*Cat 2: 1.25 < C ≤ 12.5 mg/kg bw/day*	at 250 ppm - ↓ RBC (5 %*) in females - ↑ kidney pigmentation - ↑ hematopoiesis in sternum/rib bone marrow in females
24-month oral toxicity/ carcinogenicity study in rats OECD test guideline 453, GLP Rat, Sprague-Dawley, Charles River CD 60/sex/group, oral (diet) 0, 20, 100, 500 ppm M: 1, 5, 28 mg/kg bw/ day F: 1, 7, 34 mg/kg bw/ day 1980 RAR B.6.5.1/06 M-145589-01-1	*Cat 2: 1.25 < C ≤ 12.5 mg/kg bw/day*	at 20 ppm and 100 ppm - ↑ RBC in males. The haematological changes were transient
24-month oral toxicity/ carcinogenicity study in rats OECD test guideline 453, GLP Rat, Han Wistar 50/sex/group, oral (diet) 0, 100, 500, 2500 ppm M: 4.6, 23.6, 117.6 mg/ kg bw/day F: 6.4, 33.1, 170.5 mg/ kg bw/day 2004 RAR B.6.5.1/07 M-240148-01-1	*Cat 2: 1.25 < C ≤ 12.5 mg/kg bw/day*	At 100 ppm - ↑ MetHb 62 %* in females in week 13 (% of Hb: 0.60* vs 0.37 in controls) - congestion of the spleen (minimal to slight) in males (17/20* vs 9/20 in controls) in week 52

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

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

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

Following oral administration for rats, mice and dogs in the short -term studies, the effects are consistent with effects pointing towards methemoglobinemia, leading to changes in RBC parameters and slight hemolytic anemia, increased activities of the bone marrow, liver and spleen - the organs mainly involved in the turnover of red blood cells - and compensatory hematopoiesis. Effects were most clearly seen in the 13-week rat study RAR B.6.3.2/02 (1986a) and 90-day rat study RAR B.6.3.2/06 (2002), and in the 60-day dog study RAR B.6.3.1/02 (2001). These findings were also supported by the results from the oral combined chronic toxicity/carcinogenicity studies in mice and rats. Short-term studies with the structurally and toxicologically related substance, desmedipham, have shown similar effects on haematopoietic system (see section 10, read-across justification).

The effects on blood are considered significant health effects. However, at the dose levels approximately equal to STOT RE 2 guidance values (cut-offs) haematotoxic effects were stayed rather slight or moderate and in most of cases were reversible. Reductions in haemoglobin were never ≥ 20 % and only slight or moderate decreases in Hb (up to 17 % in rats at 214 mg/kg bw) and in other red blood cell parameters were observed (i.e RBC, Hct). Increases in MetHb levels (% of Hb) in blood were minimal/slight (in rats up to 1.1 at 189 mg/kg bw vs 0.4 % of Hb in controls and in dogs 2.9 % at 118 mg/kg bw vs 0.5 % of Hb in controls, respectively) were seen. According to Solecki et al. (2005[1]), a statistically significant increase in MetHb in rodents are considered adverse and 4% of increase in methaemoglobin in dogs is considered as threshold for adversity. It should be noted that the Solecki paper considers acute exposure to MetHb inducing substances and the earliest measures are after four weeks of treatment. Slight increases in formation of Heinz bodies were also seen indicating degenerated haemoglobin. MetHb is usually rapidly reduced back to Hb and, in some cases, the appearance of Heinz bodies can be considered to be a more robust indicator of MetHb formation rather than measurements of blood MetHb concentrations as Heinz bodies are more persistent than MetHb[2]. In addition, increased plasma cholinesterase activity was observed which may be due to cholinesterase released from the hemolyzed red blood cells.

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

The haemotoxic effects caused by phenmedipham are considered a borderline case for classification. Strictly, applying the CLP guidance (CLP Guidance, 3.9.2.5.2, Haemotoxicity), none of these effects are considered significant or sufficient for classification. However, the guidance on the application of the CLP criteria also states that in the case where multiple less severe effects with regenerative capacity were observed, the

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

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

classification should apply as "Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs".

Although the severity of the haemotoxic effects represents a borderline case, multiple less severe and dose-related effects with regenerative capacity involving several organs were observed consistently in oral repeated dose toxicity studies at the dose levels approximately equal to the STOT-RE 2 guidance values. These effects are considered sufficient for classification. This conclusion is further supported by similar toxicological profile of structurally related substance desmedipham (see section 10, read-across justification). Therefore, classification of phenmedipham for STOT-RE 2 ("H373: May cause damage to organs (blood) through prolonged or repeated oral exposure") is proposed. Since studies were conducted oral route, it is not proposed to specify a route of exposure.

10.13.3 Conclusion on classification and labelling for STOT RE

Overall, in conclusion, based on haemotoxic effects seen in repeated dose toxicity studies in mice, rats and dogs, classification of phenmedipham for STOT-RE 2 ("H373: May cause damage to organs (blood) through prolonged or repeated oral exposure") is proposed. Since studies were conducted oral route, it is not proposed to specify a route of exposure.

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

Summary of the Dossier Submitter's proposal

The repeat dose toxicity of phenmedipham via the oral route has been investigated in the rat, mouse and dog. Effects indicative of haemolytic anaemia were observed in all three species. Although the effects below the guidance values did not meet any of the individual criteria listed in the Guidance on the application of the CLP criteria v. 5.0 (CLP guidance), the DS proposed classification with STOT RE 2; H373 (blood) 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.

Comments received during public consultation

Comments were provided by 3 Member State Competent Authorities (MSCAs) and 1 Industry association.

While 2 MSCAs supported STOT RE 2 (blood), 1 MSCA and the Industry association did not find the haematologic effects sufficiently adverse to meet the classification criteria.

Assessment and comparison with the classification criteria

No significant effects below the guidance values for classification were observed in the available studies with phenmedipham except for slight haematotoxicity (reduced haemoglobin (Hb), increased haemosiderin deposition in the spleen, liver and kidney, increased extramedullary haematopoiesis, increased spleen weight). A detailed summary of effects below the guidance values (extrapolated according to the Haber's rule) is provided in Tables 29 and 30 of the CLH report. Additional information can be found in

the RAR.

Haematological effects have been observed following exposure durations ranging from 4 weeks to 2 years. 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. However, haematological measurements in studies B.6.3.2/06 and B.6.5.1/07 show that the effective doses for Hb reduction are the same regardless of whether exposure duration is 1 month or 2 years (see 'Supplemental information'). Thus, the effect does not follow Haber's rule. For this reason, RAC does not consider extrapolation of the 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.

CLP provides specific guidance on classification of substances causing haemolytic anaemia. According to this guidance, if a haemolytic substance induces one or more of the serious health effects listed in the table below within the guidance values, classification is warranted. It is sufficient for classification that only one of these criteria is fulfilled. The table summarises the effects in studies with phenmedipham corresponding to the individual criteria.

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

Criterion	**Corresponding effects in studies with phenmedipham**	**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	None	–
(3) Reduction in Hb at ≥ 20 %	Maximum Hb reduction around/below 100 mg/kg bw/d by approx. 4-8 %	90-day rat study B.6.3.2/05, 1 000 ppm
(4) Reduction in functional Hb at ≥ 20 % due to a combination of Hb reduction and MetHb increase	No or only a slight increase in MetHb → Reduction in functional Hb by < 10 %	90-day rat study B.6.3.2/06, 1 000 ppm 60-day dog study 6.3.1/02, 3 000 ppm
(5) Haemoglobinuria that is not limited to the first three days of treatment in the repeated dose study in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥ 10 %)	None	–
(6) Haemosiderinuria supported by relevant histopathological findings in the kidney in combination with other changes indicating significant haemolytic anaemia	None	–

(e.g. a reduction in Hb at ≥ 10 %)		
(7) Multifocal or diffuse fibrosis in the spleen, liver or kidney	None	–
(8) Tubular nephrosis	None	–
(9) Marked increase of haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥ 10 %) in a 28-day study	No 28-day study available to see whether an increase in haemosiderosis occurs already after 28 days A possibly "marked" increase in haemosiderosis in some of the rat studies (B.6.3.1/01, /02) from ca. 100 mg/kg bw/d Hb reduction < 10 %	90-day rat studies 6.3.2/01, /02, /05, /06
(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 (not even above the guidance values except for hepatic necrosis at ca. 1 000 mg/kg bw/d in the 60-day dog study B.6.3.1/02)	90-day rat studies 6.3.2/01, /02, /04, /05, /06 8-week mouse study 6.3.1/01 60-day dog study 6.3.1/02 2-year rat studies 6.5.1/03, /05, /07

The table above shows that none of the individual criteria for classification 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.

As the haematotoxic effects are below the guidance values and do not meet the criteria for classification and there were no relevant effects in other organs, **RAC proposes no classification for STOT RE**.

Supplemental information - In depth analyses by RAC

Time dependence of haematological effects

The tables below show that the severity of Hb reduction does not depend on exposure duration, from 1 month to 2 years in the rat, and that the effective doses did not decrease with increasing study duration. The increase in methaemoglobin (MetHb) was minimal after both 1 month and 2 years.

The effect level for increased haemosiderin deposition in the liver, kidneys or spleen did not decrease from 3 months to 2 years (LOELs between 250 and 500 ppm for both study

durations; see RAR B.6.3.2/01, /02, /04, /06; B.6.5.1/03, /05, /07).

Haemoglobin reduction (compared to control) in 90-day rat study B.6.3.2/06				
	Dose	**Week 4**	**Week 8**	**Week 12**
Males	1 000 ppm (61 mg/kg bw/d)	ns	-5 %	-6 %
	3 000 ppm (189 mg/kg bw/d)	Ns	-6 %	-7 %
	10 000 ppm (636 mg/kg bw/d)	-12 %	-12 %	-9 %
Females	1 000 ppm (71 mg/kg bw/d)	-8 %	ns	-6 %
	3 000 ppm (214 mg/kg bw/d)	-17 %	-11 %	-13 %
	10 000 ppm (658 mg/kg bw/d)	-17 %	-14 %	-18 %

ns = no statistically significant difference

Methaemoglobin (% of haemoglobin) in 90-day rat study B.6.3.2/06				
	Dose	**Week 4**	**Week 8**	**Week 12**
Males	0 ppm	0.4	1.0	1.3
	1 000 ppm (61 mg/kg bw/d)	0.9*	1.1	1.0
	3 000 ppm (189 mg/kg bw/d)	1.1*	1.3	1.0
	10 000 ppm (636 mg/kg bw/d)	1.2*	1.3	1.4
Females	0 ppm	0.8	1.0	1.0
	1 000 ppm (71 mg/kg bw/d)	0.8	1.0	1.1
	3 000 ppm (214 mg/kg bw/d)	1.1	1.0	0.9
	10 000 ppm (658 mg/kg bw/d)	1.1	1.3	1.6*

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

Haemoglobin reduction (compared to control) in 2-year rat study B.6.5.1/07						
	Dose	**Week 13**	**Week 26**	**Week 52**	**Week 78**	**Week 104**
Males	500 ppm (24 mg/kg bw/d)	-4 %	ns	ns	ns	ns
	2 500 ppm	-10 %	-9 %	-3 %	-5 %	-6 %

	(118 mg/kg bw/d)					
Females	500 ppm (27 mg/kg bw/d)	ns	ns	ns	ns	ns
	2 500 ppm (171 mg/kg bw/d)	-10 %	-12 %	-11 %	-9 %	-9 %

ns = no statistically significant difference

Methaemoglobin (% of haemoglobin) in 2-year rat study B.6.5.1/07

	Dose	**Week 13**	**Week 26**	**Week 52**	**Week 78**	**Week 104**
Males	Control	0.4	0.6	0.7	0.6	0.6
	2 500 ppm (118 mg/kg bw/d)	0.7*	0.8*	0.8	0.8*	0.7
Females	Control	0.4	0.5	0.7	0.5	0.4
	2 500 ppm (171 mg/kg bw/d)	0.8*	0.9*	0.9*	0.9*	0.9*

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

10.14 Aspiration hazard

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Summary of relevant studies from the Renewal Assesment Report on degradation is reported below (RAR annexed to this CLH proposal). Only relevant and valid studies for the proposed classification of phenmedipham have been included from the RAR. Studies for soil degradation and adsorption to soil are only summarised briefly in sections 11.1.4.3 and 11.3 as they are less relevant for the classification of phenmedipham.

Half-lives of phemedipham in simulation tests in water/sediment systems represent dissipation in water phase and in total system. The DT50 values were calculated from the resulting kinetic parameters according to FOCUS. Generally, the calculations were made by using single first-order (SFO) model and where phenmedipham showed non-SFO behaviour first order multi-compartment (FOMC) model was used instead.

Table 31: Summary of relevant information on rapid degradability

Method	**Results**	**Remarks**	**Reference**
Ready biodegradability: - study followed "Modified MITI-Test (I)" OECD 301C GLP compliant	**Biodegradation** 34.1 % (ThOD) after 14 days 54.3 % (DOC) after 14 days	Test substance was not totally soluble during the study.	1989 RAR B.8.2.2.1/01 M-145994-01
Ready biodegradability:	**Biodegradation** 23-31 % (ThOD) after 35 days		1985 RAR B.8.2.2.1/03

Method	Results	Remarks	Reference
- study followed "Modified MITI-Test (I) OECD 301C No GLP compliant			M-146460-01
Hydrolysis: - study followed OECD 111 GLP compliant	**DT_{50} obtained at 25°C (^{14}C-amino phenol)** pH 4 = 259 d pH 5 = 47 d pH 7 = 12 h pH 9 = 7 min	DT_{50} values assuming first order kinetics. Degradation products are MHPC and m-toluidine	2003 RAR B.8.2.1.1/03 M-215907-01
Hydrolysis: - study followed OECD 111 GLP compliant	**DT_{50} values obtained at 24-25°C (^{14}C-amino phenol)** pH 4 = 144 d pH 5 = 19 d pH 7 = 3 h pH 9 = 2 min **DT_{50} values obtained at 24-25°C (^{14}C-methyl aniline)** pH 4 = 140 d pH 5 = 18 d pH 7 = 3 h pH 9 = 2 min	DT_{50} values assuming first order kinetics. Degradation products are MHPC and m-toluidine	2004 RAR B.8.2.1.1/04 M-493396-01-1
Hydrolysis: - OECD 111 GLP compliant	**DT_{50} values obtained at 20°C (^{14}C-methyl aniline)** pH 4.0 = 1011 d pH 4.5 = 262.5 d pH 5.0 = 91.2 d pH 5.5 = 31.7 d pH 6.0 = 11.3 d pH 6.5 = 3.3 d pH 7.0 = 1 d pH 7.5 = 8 h pH 8.0 = 3 h	DT_{50} values assuming first order kinetics. Degradation products are MHPC and m-toluidine	2015 RAR B.8.2.1.1/05 M-533313-01-1
Hydrolysis: - study followed OECD 111 GLP compliant	**<u>Degradate MHPC</u>** Hydrolytically stable at pH 4, 5, 7 and 9 after 120 hours at 50°C.	The study was conducted for degradation product MHPC	2003 RAR B.8.2.1.1/06 M-227344-01
Hydrolysis: - study followed OECD 111 GLP compliance	**<u>Degradate MHPC</u>** Hydrolytically stable at pH 4, 5, 7 and 9 after 120 hours at 50°C.	The study was conducted for degradation product MHPC	2004 RAR B.8.2.1.1/07 M-493394-01-1
Inherent biodegradability: - study followed "Modified MITI-Test (II)" OECD 302C GLP compliant	**Inherent biodegradation** 39.5 % (ThOD) after 28 days 19.2 % (DOC) after 28 days	Test substance was not totally soluble during the study.	1990 RAR B.8.2.2/02 M-146096-01
Aquatic simulation test: - OECD 309	**DT_{50} (water phase)** pH 7.8 = 0.04 d (^{14}C-methyl aniline) pH 7.8 = 0.01-0.04 d (^{14}C-amino phenol)	The study was conducted at 20.9 °C. DT_{50} values calculated	2013 RAR B.8.2.2.2/01 M-476398-01-1

Method	Results	Remarks	Reference
GLP compliant	**Mineralisation to CO_2** 15 % AR after 63 days (^{14}C-methyl aniline) 11.9-13.2 % AR after 63 days (^{14}C-amino phenol) **<u>Degradate m-toluidine</u>** DT_{50} = 47.6 d **<u>Degradate MHPC</u>** DT_{50} = 499 d	according to FOCUS kinetics (RAR B.8.2.2.2/02).	2014 RAR B.8.2.2.2/02 M-496963-01-1
Water/sediment simulation test: - study followed OECD 308 GLP compliant	**DT_{50} (water phase)** **(^{14}C-amino phenol)** pH 6.9 = 0.16 d pH 7.0 = 0.11 d **DT_{50} (water-sediment system)** **(^{14}C-amino phenol)** sandy loam silt = 0.0069 d sand = 0.15 d **Mineralisation to CO_2** 29.8-34.1 % AR after 126 days (^{14}C-amino phenol) 12.6-13.8 % AR after 70 days (^{14}C-methyl aniline) **NER % in sediment** 50.8-55.3 % AR after 126 days (^{14}C-amino phenol) 69.7-73.4 % AR 70 days (^{14}C-methyl aniline) **<u>Degradate MHPC</u>** DT_{50} = 11.6-13.8 d (water) DT_{50} = 9.2-15.3 d (water-sediment)	The study was conducted at 20°C. Two different water-sediment systems from Germany were used (sandy loam silt and sand sediment types). DT_{50} values calculated according to FOCUS DegKinetics (RAR B.8.2.2.3/01).	1996 RAR B.8.2.2.3/02 M-147079-01-1 2014 RAR B.8.2.2.3/01 M-501964-01-1
Water/sediment simulation test: - OECD 308 No GLP compliant	**DT_{50} (water phase)** **(^{14}C-amino phenol)** pH 6.0-6.5 = 0.012 d **DT_{50} (water-sediment system)** **(^{14}C-amino phenol)** pH 6.0-6.5 = 0.023 d **Mineralisation to CO_2** 13.2 % AR after 84 days	The study was conducted at 22°C. Water-sediment system from Germany were used (natural pond). DT_{50} values calculated according to FOCUS DegKinetics (RAR B.8.2.2.3/01).	1984 RAR B.8.2.2.3/03 M-145920-01-1 2014 RAR B.8.2.2.3/01 M-501964-01-1
Water/sediment simulation test: - OECD 308 No GLP compliant	**DT_{50} (water phase)** **(^{14}C-amino phenol)** pH 6.8 = 0.35 d pH 7.4 = 0.27 d pH 8 = 0.24 d **DT_{50} (water-sediment system)** **(^{14}C-amino phenol)** sandy loam = 0.35 d silt loam = 0.0001 d	The study was conducted at 20°C. Three different water-sediment systems from the USA were used (silt loam, sand, sandy loam). DT_{50} values calculated according to FOCUS	2005, 2006 RAR B.8.2.2.3/04 M-210543-01-1 M-493403-02-1 2014 RAR B.8.2.2.3/01 M-501964-01-1

Method	Results	Remarks	Reference
	sand = 0.0007 d **Mineralisation to CO_2** 27.8-31.2 % AR after 127 days (^{14}C-amino phenol) 14.3-29.8 % AR after 100 days (^{14}C-methyl aniline) **NER % in sediment** 38.6-44.2 % AR after 126 days (^{14}C-amino phenol) 34.0-52.1 % AR after 100 days (^{14}C-methyl aniline) **<u>Degradate MHPC</u>** DT_{50} = 8.8-20.2 d (water) DT_{50} = 8.7-21 d (water-sediment)	DegKinetics (RAR B.8.2.2.3/01).	
Water/sediment simulation test: - OECD 308 GLP compliant	**DT_{50} (water phase) (^{14}C-amino phenol)** pH 7.8 = 0.12 d pH 8.4 = 0.12 d **DT_{50} (water phase) (^{14}C-methyl aniline)** pH 7.8 = 0.13 d pH 8.4 = 0.18 d **DT_{50} (water-sediment system) (^{14}C-amino phenol)** silt clay loam = 0.09 d sandy loam = 0.12 d **DT_{50} (water-sediment system) (^{14}C-methyl aniline)** silt clay loam = 0.14 d sandy loam = 0.21 d **Mineralisation to CO_2** 24.1-24.2 % AR after 98 days (^{14}C-amino phenol) 13-54.8 % AR after 98 days (^{14}C-methyl aniline) **NER % in sediment** 65.6-68.6 % AR after 98 days (^{14}C-amino phenol) 31.8-67.7 % AR after 98 days (^{14}C-methyl aniline) **<u>Degradate MHPC</u>** DT_{50} = 8.7-13.3 d (water) DT_{50} =13.3-17.6 d (water-sediment) **<u>Degradate m-toluidine</u>** DT_{50} = 0.75-4.4 d (water) DT_{50} = 1.5-4.7 d (water-sediment)	The study was conducted at 20.7°C. Two different water-sediment systems from Switzerland (sandy loam) and France (silt clay loam) were used. DT_{50} values calculated according to FOCUS DegKinetics (RAR B.8.2.2.3/01).	2012 RAR B.8.2.2.3/05 M-459409-01-1 2014 RAR B.8.2.2.3/01 M-501964-01-1

Method	Results	Remarks	Reference
Photolysis: - study followed OECD 316 GLP compliant	Photolytically stable at 23°C	The study design corresponded to 35.4 days in natural sunlight.	1992 RAR B.8.2.1.2/01 M-146124-01-1
Photolysis: - OECD 101 (1981) - OECD 316 (2008) GLP compliant	The degradation of phenmedipham was 3.5-4.8 % after a maximum irradiation period of 500 min at 25±1°C. Quantum yield of Φ = 0.000136	The calculated quantum yield translates into estimated photolytic half-life of about 36 to 77 days.	2013 RAR B.8.2.1.2/02 M-473897-01-1

11.1.1 Ready biodegradability

Study 1 ─ Phenmedipham

RAR B.8.2.2.1/01 (1989)

Phenmedipham (purity 99.8 % w/w) was investigated for its biodegradability in test concentration of 25 mg/250 mL in the Modified MITI-Test (I) following the OECD test guideline 301C during 14 days in three replicates. The biodegradation of the test article was followed by exposing it to microorganisms from the secondary effluent of a domestic waste-water sewage plant. Results were compared with those obtained with reference compound aniline, tested simultaneously under the same conditions. Temperature ranged from 21.5-24°C and was controlled during the study.

The test was finished after 14 days since plateau phase was obtained. The ThOD-values for phenmedipham and aniline were calculated to be 1.76 mg O_2/mg and 2.41 mg O_2/mg, respectively. 34.1 % (BOD/ThOD) or 54.3 % (BOD/DOC) of the parent compound was degraded within this time. Aniline was degraded after 7 and 14 days to 70.1 % and 71.6 % (BOD/ThOD), respectively.

RMS considered this study valid even though the test substance was not totally soluble during the study. At the end of the test, it was still not completely dissolved and a white precipitation was observed. The test was still considered valid in the RAR, since the difference in the extreme replicates was within 20 % at the plateau and at the end of the test for phenmedipham and just above 20 % for the reference compound aniline. Also the time window of 10 days for 60 % degradation was achieved with aniline.

Study 2 ─ Phenmedipham

RAR B.8.2.2.1/03 (1985)

The ready biodegradability of phenmedipham (purity 99.98 %) was studied following a Modified Miti-Test (I) following the OECD test guideline 301C with nominal concentration of the test substance of 100 mg/L using an activated sludge. A 2 litre volume of inoculated test medium was prepared containing mineral salts and the activated sludge inoculum (suspended solid concentration 30 mg/L). The study was performed with duplicate respirometric flasks in a water bath at 24 °C.

The amount of oxygen generated by electrolysis was recorded automatically, over a period of 28 days extended to 35 days after treatment, in each flask. Oxygen uptake values for phenmedipham were corrected for the blank and then expressed as a percentage of their respective theoretical oxygen demands (ThOD).

The degradation of phenmedipham after blank correction was between 23 % and 31 % of its ThOD after 35 days. Degradation of the phenmedipham started on day 16 in both flasks. A plateau had not been reached by day 35. Oxygen consumption in the abiotic control containing phenmedipham was equivalent to 27 % of the ThOD by day 35. The cumulative uptake of oxygen in abiotic control (31 mg) at day 35 was almost equivalent to that of blank (35-37 mg). The oxygen consumption was noted mainly between day 8 and day 19 in abiotic control whereas in the blank it was between day 2 and day 12.

The results of the test were complicated by an inhibitory effect of phenmedipham on nitrification. Nitrogen added in the mineral salt medium and nitrogen released from the phenmedipham during degradation was present as ammonia in one of the flasks containing phenmedipham, mineral salts medium and inoculum, whereas 75% of this nitrogen was oxidised to nitrite and 20% was oxidised to nitrate in a second flask with the same content. Nitrogen in the flasks containing the blank and in those containing the reference mixture was completely oxidised to nitrate. In biotic control 99 % of nitrogen was present as ammonia at the end of the study.

The reference substance, sodium benzoate, started to degrade on day 1 of the test. Degradation of the sodium benzoate, after blank correction, had reached 74 % and 75 % by day 11. Thus, as evaluated using EEC criteria, the inoculum used in this test had a normal biodegradation activity.

This study is considered valid in the RAR.

11.1.2 BOD_5/COD

No information available in the RAR.

11.1.3 Hydrolysis

Study 1 ─ Phenmedipham

RAR B.8.2.1.1/03 (2003)

The abiotic hydrolysis was investigated following the OECD test guideline 111 in a sterile aqueous buffer at pH 4, 5, 7 and 9 following application of [amino phenol-UL-^{14}C] phenmedipham at nominal concentration of 3 mg/L following incubation at 25±1ºC in the dark. Phenmedipham was hydrolysed at pH 4, 5, 7 and 9 to MHPC. The half-life of phenmedipham in aqueous buffers was calculated assuming first order kinetics.

The recovery of radioactivity was good, with all samples being within an acceptable range of 90 to 110 % of the applied radioactivity. No other hydrolysis products of phenmedipham than MHPC were found and no volatile components were formed at any pH. The hydrolytic degradation of phenmedipham was strongly depended on the pH of the solution. At pH 4 the maximum percentage of hydrolysed MHPC was 8.0 % of the AR after 672 hours, and at pH 5 38.0 % of AR after 720 hours. At pH 7 and 9 the phenmedipham was totally hydrolysed to MHPC after 72 hours and 30 minutes, respectively.

Table 32: Half-lives and rate constants of phenmedipham in buffer solutions at 25 °C

pH	k	R^2	DT_{50}	DT_{90}	MHPC (%)
4	0.0027 d^{-1}	0.9726	259 days	861 days	6.8 %
5	0.0148 d^{-1}	0.9958	47 days	156 days	37.5 %
7	0.0588 h^{-1}	0.9922	12 hours	39 hours	98.8 %
9	0.0981 min^{-1}	0.9860	7 min	24 minutes	94.8 %

This study is considered valid in the RAR.

Study 2 ─ Phenmedipham

RAR B.8.2.1.1/04 (2004)

The abiotic hydrolysis of phenmedipham was investigated following the OECD test guideline 111 in a sterile aqueous buffer at pH 4, 5, 7 and 9 following application of [amino phenol-UL-^{14}C] and [methyl aniline-UL-

^{14}C]phenmedipham at the test concentration of 0.9 mg/L. The test vessels were incubated at 24-25°C in the dark.

Phenmedipham was hydrolysed to form two degradation products, MHPC and m-toluidine. At all pH mean material balances of samples were above 96.4 % AR. At pH 4 maximum occurrences of MHPC and m-toluidine were 14.2 % AR and 11.2 % AR, respectively, at day 30. At pH 5 maximum occurrences of MHPC and m-toluidine were 62.1 % AR and 60.5 % AR, respectively, at day 30. At pH 7 and 9 the phenmedipham was totally hydrolysed to MHPC and m-toluidine after 30 days.

The hydrolytic degradation of phenmedipham was strongly depended on the pH of the solution with slower degradation observed at lower pH. The mean calculated half-lives of phenmedipham assuming first order kinetics were 142 days, 18.5 days, 3 hours and 2 minutes at pH 4, 5, 7 and 9, respectively.

Table 33: Degradation kinetics and half-lives of phenmedipham in sterile buffer

Buffer (pH)	Label	DT_{50}	DT_{90}	Degradation rate	Correlation coefficient
4	amino phenol	144 days*	477 days*	0.0048 d^{-1}	0.9995
	methyl aniline	140 days*	465 days*	0.0050 d^{-1}	0.9991
5	amino phenol	19 days	62 days*	0.0372 d^{-1}	0.9989
	methyl aniline	18 days	59 days*	0.0390 d^{-1}	0.9976
7	amino phenol	3 hours	11 hours	4.9676 d^{-1}	0.9974
	methyl aniline	3 hours	11 hours	5.2764 d^{-1}	0.9976
9	amino phenol	2 minutes	6 minutes	525.42 d^{-1}	0.9924
	methyl aniline	2 minutes	8 minutes	413.32 d^{-1}	0.9902
* extrapolated values obtained for pHs 4 and 5 due to limited degradation					

This study is considered valid in the RAR. RMS mentioned that the temperature was not maintained within ±0.5°C during the study, since the temperature of the samples was 25±1°C for pH 7 and pH 9 samples and at 24±2°C for pH 4 and pH 5 samples. However, these deviations were not considered to affect the degradation rates.

Study 3 – Phenmedipham

RAR B.8.2.1.1/05 (2015)

The hydrolysis of phenmedipham was investigated following the OECD test guideline 111 at 20°C in sterile aqueous buffer solutions at pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 in the dark for 30 days. The study was performed with [amino-phenol -UL-^{14}C]phenmedipham at nominal test concentration of 2.73 mg/L.

The mean calculated half-lives of phenmedipham ranged from 1011 days at pH 4.0 to 3.0 hours at pH 8.0. Phenmedipham was hydrolytically comparatively stable under acidic conditions (pH 4.0 and 4.5), labile in slightly acidic (5.0, 5.5 and 6.0) and neutral conditions (pH 6.5), and undergoes rapid hydrolysis above pH 7.

Table 34: Degradation kinetics and half-lives of phenmedipham

Test No./ Temperature	**pH**	**Single first order Kinetics (SFO)**			
		DT_{50} (days)	**DT_{90} (days)**	**Chi^2 Error (%)**	**R^2**
Main test / 20 °C	**4.0**	1011	3359	1.77	0.06

Main test / 20 °C	**4.5**	263	872	1.31	0.55
Main test / 20 °C	**5.0**	91	303	1.57	0.90
Main test / 20 °C	**5.5**	32	105	1.70	0.98
Main test / 20 °C	**6.0**	11	37	1.83	0.99
Main test / 20 °C	**6.5**	3.3	11.0	2.70	0.99
Main test / 20 °C	**7.0**	24 hours	78 hours	2.40	0.99
Main test / 20 °C	**7.5**	8 hours	28 hours	3.01	0.99
Main test / 20 °C	**8.0**	3 hours	9 hours	3.10	0.99

This study is considered valid in the RAR. RMS mentioned that the temperature was within 20.2-22.6°C during the study. This deviation from the guideline (recommended within ±0.5°C) was not considered to affect the degradation rates.

Study 4 ─ degradate MHPC

RAR B.8.2.1.1/06 (2003)

The abiotic hydrolysis of the degradate MHPC was investigated following the OECD test guideline 111 in sterile aqueous buffers at pH 4, 5, 7 and 9 following application of [ring-UL-14C]MHPC and incubation at 50°C in the dark. MHPC was stable under sterile aqueous conditions at pH 4, 5, 7, and 9 for 0, 2.4, and 120 hours at 50°C at the nominal concentration of 5 mg/L. The recovery of radioactivity in the test was good, with all samples being within an acceptable range of 90 to 110 % of the applied radioactivity.

This study is considered valid in the RAR.

Study 5 ─ degradate MHPC

RAR B.8.2.1.1/07 (2004)

The abiotic hydrolysis of MHPC was investigated following the OECD test guideline 111 in sterile aqueous buffers at pH 4, 5, 7 and 9 following application of [ring-UL-14C]MHPC and incubation at 50°C in the dark. As MHPC showed less than 10 % hydrolysis under sterile aqueous conditions at pH 4, 5, 7, and 9 for 0, 2.4, and 120 hours at 50°C at the mean concentration of 1 mg/L, it can be considered to be hydrolytically stable. The recovery of radioactivity in the test was good, with all samples being within an acceptable range of 90 to 110 % of the applied radioactivity.

This study is considered valid in the RAR.

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Not relevant for this classification proposal.

11.1.4.2 Inherent and enhanced ready biodegradability tests

Study 1 ─ Phenmedipham

RAR B.8.2.2.1/02 (1990)

The inherent biodegradability was studied using phenmedipham (purity >99.5 %) according to the Modified MITI-Test (II) OECD 302C during 28 days. At the beginning of the test, the test article seemed to be insoluble and at the end of the test, a white precipitation was observed.

The ThOD-values for phenmedipham and aniline were calculated to be 1.76 mg O_2/mg and 2.41 mg O_2/mg, respectively. After 28 days of exposure, the rate of degradation amounted to 39.5 % when estimated using the ratio BOD/ThOD, whereas a degradation of 19.2 % was observed when estimated using the DOC-values. The standard compound, aniline, was readily degraded after 7 and 28 days by 69.8 % and 72.9 % (BOD/ThOD), respectively.

RMS considered this study valid in the RAR even though the test substance was not totally soluble during the study.

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

Surface water degradation

Study 1 ─ Phenmedipham

RAR B.8.2.2.2/01 (2013)

The Degradation of [methyl aniline-UL-^{14}C]phenmedipham (purity > 99 %) and [amino phenol-UL-^{14}C]phenmedipham (purity > 99 %) were studied according to the OECD test guideline 309 simulation in surface water under aerobic conditions in the dark for 63 days at 20.9±0.2°C. Natural water (pH 7.8) from a pond system, that was located far from human activity and received no effluent discharges, was used as a test water. Application rates of 0.1 and 0.01 mg/L were applied for the low and the high concentration samples, respectively. Experiment was also performed in addition under sterile condition at the high concentration (0,01 mg/L). Low concentration samples (0.1 mg/L) were prepared only with [amino phenol-UL-^{14}C]phenmedipham.

Standard conditions were maintained during study and the test water was microbial active. The complete material balances found at all sampling intervals, except at day 63 sterile samples treated with the methyl aniline label, demonstrated that there was no significant loss of radioactivity. Tests showed that the incomplete mass balance at day 63 sterile samples could be attributed to presence of mercury chloride used for sterilisation and subsequent precipitation of a complex between mercury and m-toluidine. Biological activity of the test water was confirmed by the degradation of reference substance UL-^{14}C-benzoic acid within 14 days of incubation.

The mineralisation (CO_2) was 15.0 % AR at day 63 for [methyl aniline-UL-^{14}C]phenmedipham. In the sterile condition mineralisation to CO_2 was 2.5 % at day 63. The amounts of phenmedipham (methyl aniline label) decreased very fast in all systems. After 2 and 6 hours of incubation, the test item concentration decreased to non-detectable amounts in non-sterile and sterile conditions. Degradate m-toluidine was observed under all conditions in methyl aniline labelled phenmedipham. M-toluidine reached a maximum occurrence of 103.4 % AR at 6 hours and decreased to 82.7 % AR at day 14 in high concentration samples. In sterile samples m-toluidine reached a maximum of 93.7 % AR at day 1 and decreased to 87.2 % AR at day 14.

The mineralisation (CO_2) was 11.9 % and 13.2 % AR at day 63 for [amino phenol-UL-^{14}C]phenmedipham. In the sterile condition CO_2 detected was 0.2 % AR at day 63 showing the need for microbial degradation for mineralisation. The amounts of phenmedipham (amino phenol label) decreased to non-detectable concentrations after 6 hours in high concentration and after 2 hours in low concentration and in sterile systems. Degradate MHPC was observed under all conditions in amino phenyl labelled phenmedipham. The amount of MHPC increased from 4.8 % AR and non-detectable amounts at the start of the incubation to 103.2 and 102.5 % AR at 6 hours and 2 hours in high concentration and low concentration test systems, respectively, and remained constant until day 42. At day 63 the amount of MHPC was decreased to 58.1 and 90.3 % AR in high concentration and low concentration, respectively. Similar occurrences for MHPC were observed in sterile test systems except around 100 % AR at the end of the study.

Rapid primary degradation of phenmedipham to MHPC and m-toluidine in sterile systems, corresponding to the observations in the abiotic hydrolysis tests (Chapter 11.1.3), confirms that phenmedipham was rapidly hydrolysed abiotically under the test conditions. The further degradation of MHPC was slower and no significant amounts of radioactive CO_2 were found in the sterilised systems for both labels, compared to the biotic test. It is therefore concluded that phenmedipham in the natural surface water systems was readily converted to MHPC by abiotic processes, but further degradation proceeded mainly by microbiological processes.

Kinetic analysis of the residue data of phenmedipham and its degradation products MHPC and m-toluidine was performed with KinGUI 2 according to FOCUS kinetics (RAR B.8.2.2.2/02, 2014). SFO DT_{50} value for methyl aniline labelled phenmedipham was 0.04 days and SFO DT_{50} value for amino phenol labelled phenmedipham was 0.01 days and 0.04 days for low and high concentrations, respectively. SFO DT_{50} value of 499 days was evaluated for MHPC and SFO DT_{50} value of 47.6 days for m-toluidine. The half-lives in water simulation test are mainly considered to represent primary degradation of phenmedipham.

This study is considered valid in the RAR.

Water-sediment degradation

Study 1 ─ Phenmedipham

RAR B.8.2.2.3/02 (1996)

The degradation of [amino phenol-UL-^{14}C]phenmedipham (purity >99 %) and [methyl aniline-UL-^{14}C]phenmedipham (purity 97 %) was investigated in two different water/sediment systems (sandy loam silt and sand) from Germany under aerobic conditions over a period of 126 days at 20°C with the application rate of 0.94 kg/ha. Thus, the initial concentration of phenmedipham was 0.32 mg/L. The water pH in the systems were 6.9 and 7.0. The study was performed in line with OECD test guideline 308.

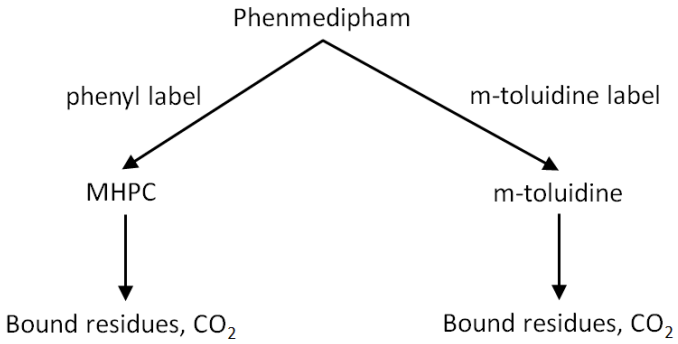
The concentration of [amino phenol-UL-^{14}C]phenmedipham was decreased rapidly in both water/sediment systems, resulting in a total concentration of the substance of 2.4-9.4 % in the system after 2 days and <0.5 % after 126 days. The concentration of phenmedipham in sediment was maximally 8.5-9.4 % of AR in the systems. The primary degradate was MHPC which reached its maximum concentration of 60-70 % within 1 to 2 days after application and declined to approximately 1 % at day 126 in both systems. After day 14, the small amounts of MHPC were mainly found in the sediment. No other degradates could be identified.

Mineralisation to CO_2 was 29.8-34.1 % at day 126 during the degradation of amino-phenyl labelled phenmedipham. CO_2 formed was 12.6-13.8 % at day 70 during the degradation of methyl aniline labelled phenmedipham. Non-extractable residues in the sediment after 126 days were 50.8-55.3 % AR for amino-phenyl labelled phenmedipham and after 70 days 69.7-73.4 % AR for methyl aniline labelled phenmedipham.

In sterile systems the main degradate MHPC was found at 41-51 % AR at the end of the study. This confirms that phenmedipham is at least partly hydrolysed abiotically under these conditions. The further degradation of MHPC was much slower and no significant amounts of radioactive CO_2 were found in the sterilised systems. It is therefore concluded that phenmedipham in the natural water/sediment systems was readily converted to MHPC by abiotic processes, but further degradation proceeded mainly by microbiological processes.

Half-lives were evaluated according to FOCUS DegKinetics Report (RAR B.8.2.2.3/01, 2014).

Figure 2: Following degradation scheme was implemented for phenmedipham in the RAR.



The dissipation half-lives in water phase for [Amino phenol-UL-14C]phenmedipham were FOMC DT_{50} of 0.16 and SFO DT_{50} 0.11 days and in total system FOMC DT_{50} of 0.069 and 0.15 days in sandy loam silt and sand systems, respectively. The half-lives of MHPC were SFO DT50 of 9.2-15.3 days in total system and SFO DT_{50} values of 11.6-13.8 days in water phase.

RMS considered this study valid even though the mean total recovery was 81.6 at day 126 in one system. According to the OECD test guideline 308 the recovery should be 90-110 % for the labelled substances, but results in both systems were quite similar so this study was considered valid in the RAR.

Study 2 – Phenmedipham

RAR B.8.2.2.3/03 (1984)

The degradation of [amino phenol-UL-^{14}C]phenmedipham (purity >94 %) was studied in a water/sediment system from a natural pond in Germany, applied at a field rate of 4.9 kg a.s./ha. One replicate of each system was incubated at 22±2°C for 84 days. This study was conducted according to the OECD test guideline 308.

Practically no mineralisation to CO_2 (<1 %) was evolved during the first 21 days, rising to 13.2 % at the end of the study period (84 days). Main degradate detected was MHPC, which represented 72.5 % AR after 2 days. At the end of the test period it accounted for 0.3 %. No other distinct degradates were detected. Bound residues rose to 56.9 % after 42 days and represented 74.8 % after 84 days. The recoveries ranged from 72 to 100 %.

Table 35: Degradation of phenmedipham.

Day	$^{14}CO_2$	phenmedipham			MHPC			Origin*	Others**	Bound Residues	Total
		water	sediment	total	water	sediment	total				
0.02	-	39.4	12.2	51.6	6.7	0.5	7.2	10.0	3.1	0.1	72.0
2	<0.1	2.7	2.4	5.1	69.0	3.5	72.5	5.8	3.9	0.1	87.4
7	0.1	3.5	1.4	4.9	56.7	6.9	63.6	8.6	9.2	1.5	87.9
14	0.8	<0.1	1.2	1.2	53.3	8.2	61.5	8.8	8.3	10.8	91.4
21	0.9	2.1	1.7	3.8	50.3	1.9	52.2	12.4	8.5	4.0	81.8
42	7.2	<0.1	<0.1	<0.1	8.6	1.2	9.8	10.7	9.2	56.9	93.8
63	11.3	1.4	0.4	1.8	2.8	0.9	3.7	7.7	5.8	64.7	94.8
84	**13.2**	<0.1	0.4	0.4	<0.1	0.3	0.3	5.8	3.1	74.8	97.6

* Origin - fraction not mobile in TLC

** TLS scans outside distinctive spots

The half-lives were later calculated following the FOCUS degKinetics (RAR B.8.2.2.3/01, 2014). [Amino phenol-UL-^{14}C]phenmedipham half-life in total system was FOMC DT_{50} of 0.023 days and dissipation half-life was FOMC DT_{50} of 0.012 days in water phase.

RMS considers this study valid in the RAR even though the total recovery was less than 80 % at the first sampling point and no explanation was given. Also only one replicate flask was analysed per sampling point.

Study 3 ─ Phenmedipham

RAR B.8.2.2.3/04 (2005 & 2006)

The degradation of phenmedipham was investigated according to the OECD test guideline 308 in three freshwater water/sediment systems (silt loam, sand and sandy loam) from the USA using two labels, [amino phenol-UL-^{14}C]phenmedipham (purity 98.02 %) and [methyl aniline-UL-^{14}C]phenmedipham (purity 99 %), at 20°C in the dark for up to 127 days. A study application rate of 0.1 mg/L was applied.

Phenmedipham degraded very fast and MHPC and m-toluidine were the major degradation products. The concentration of [amino phenol-UL-14C]phenmedipham was below 3.1 % AR in total system after 7 days in each test system and the concentration of [methyl aniline-UL-14C]phenmedipham was below 7.1 % AR in water phase in each test system after 2 days.

MHPC reached the maximum amount of 84.6 % of AR in water phase of sandy loam system at day 2 and 11.3 % of AR in sediment of silt loam system at day 7. M-toluidine reached the maximum amount of 67.4 % of AR in water phase of silt loam system at day 2. The results of the abiotic test systems were similar to those of the biotic test system with a little bit slower degradation of phenmedipham and formation and degradation of MHPC in abiotic test systems.

After 127 days mineralisation to CO_2 was 31.2 %, 31.1 % and 27.8 % AR in silt loam, sand and sandy loam test systems in amino phenol labelled phenmedipham, respectively. In proportion, non-extractable residues in sediment were 38.6 %, 42.2 % and 44.2 % AR in silt loam, sand and sandy loam test systems.

After 100 days for methyl aniline labelled phenmedipham, mineralisation to CO_2 was 29.28 %, 24.11 % and 14.26 % AR in silt loam, sand and sandy loam test systems, respectively. In proportion, non-extractable residues in sediment were 52.1 %, 34.0 % and 46.7 % AR in silt loam, sand and sandy loam test systems.

The half-lives for [amino phenol-UL-14C]phenmedipham were later calculated following the FOCUS degKinetics (RAR B.8.2.2.3/01, 2014). The half-lives in total system were FOMC DT_{50} of 0.0001 and 0.0007 days in silt loam and sand systems, respectively, and SFO DT_{50} of 0.35 days in sandy loam system. The dissipation half-lives in water phase were SFO DT_{50} 0.27, 0.24 and 0.35 days in silt loam, sand and sandy loam systems, respectively.

The half-lives for MHPC were calculated between SFO DT_{50} of 8.7-21 days in total system and SFO DT_{50} of 8.8-20.2 days in water phase. This study is considered valid in the RAR.

Study 4 ─ Phenmedipham

RAR B.8.2.2.3/05 (2015)

The aerobic degradation of [amino phenol-UL-14C]phenmedipham (purity >98 %) and [methyl aniline-UL-14C]phenmedipham (purity >99 %) was investigated according to the OECD test guideline 308 in a river and a pond water-sediment systems from Birs river (sandy loam), Switzerland and Biederthal pond (silt clay loam), France at 20.7±2°C in the dark for 98 days. The nominal application rate was based on the maximum field application rate of 1 kg a.s./ha. The mean total recovery of radioactivity in the individual test vessels ranged from 90.4 to 100.0%.

[Amino phenol -UL-14C]phenmedipham

Phenmedipham was completely dissipated after day 1 in the water phase and it was not detected in the sediment of test systems at any sampling point. The only major degradate was identified as MHPC. In the water phase, MHPC reached 79.8 % AR at day 1 decreasing to 3.5 % AR at day 40 in the river system.

Corresponding values for the pond were 92.8 % AR and 14.2 % AR. In the sediment of the river test systems, MHPC reached a maximum amount of 18.7 % AR at day 14 and decreased to 5.1 % AR at day 98. In the pond, MHPC reached maximum mean amount of 13.9 % AR at day 7 and decreased to 1.3 % AR at day 98. In the total system, MHPC reached a maximum concentration of 91.0 % at day 1 and continuously decreased to 5.1 % AR at in the river system at day 98. In the pond, MHPC reached 97.1 % at day 1 decreasing to 1.3 % AR at day 98.

Mineralisation to CO_2 was 24 % and 24.2 % AR for phenmedipham in the river and the pond test systems after 98 days, respectively. Non-extractable residues in sediment were, in proportion, 65.6 % and 68.6 % AR after 98 days. Two additional degradation products were identified in the pond system, namely 3-[(methoxycarbonyl) amino]phenyl(3-hydroxyphenyl)carbamate (M1) and 3-aminophenol (M2). Both reached maximum amounts of 0.5% AR.

Half-lives were later calculated following the FOCUS degKinetics (RAR B.8.2.2.3/01, 2014). The half-lives for amino phenol labelled phenmedipham were SFO DT_{50} of 0.12 days in the total system and in the water phase in the Birs river system, respectively. For Biederthal pond the half-life was SFO DT_{50} of 0.09 days in total system and SFO DT_{50} of 0.12 days in water phase.

Degradate MHPC had half-lives SFO DT_{50} of 13.3 and 17.6 days in total system in Birs river and Biederthal pond, respectively. In water phase half-lives were SFO DT_{50} of 8.7 and 13.3 days in Birs river and Biederthal pond, respectively.

[Methyl aniline-UL-14C]phenmedipham

In the water phase of the river and the pond test systems, the amount of phenmedipham decreased from 84.8 and 98.3 % AR at the beginning of the test to not detectable amounts from day 3 onwards. One major degradate m-toluidine was detected reaching 73.2 % AR at day 1, decreasing rapidly to 7.4 % AR by day 7 and representing 2.0 % at day 40. In the sediment of the pond and river test systems, m-toluidine reached maximum amounts of 4.4 and 2.9 % on days 3 and 7, respectively. Other degradation products observed were all less than 8.5 % AR at any sampling point and represented total 4.6 % AR at day 40.

Mineralisation to CO_2 accounted for 54.8 and 13 % AR for phenmedipham in the river and the pond systems at day 98, respectively. Non-extractable residues in sediment were, in proportion, 31.8 % and 67.7 % AR after 98 days.

The degradation half-life for methyl aniline labelled phenmedipham was SFO DT_{50} of 0.21 days in total system. The dissipation half-life was SFO DT_{50} of 0.18 days in water phase. For Biederthal pond the half-life was SFO DT_{50} of 0.14 days in total system. The dissipation half-life was SFO DT_{50} of 0.13 days in water phase.

Degradate m-toluidine had half-lives SFO DT_{50} of 1.5 and 4.7 days in total system in Birs river and Biederthal pond, respectively. In water phase dissipation half-lives were FOMC DT_{50} of 0.75 days and SFO DT_{50} of 4.4 days in Birs river and Biederthal pond, respectively.

This study is considered valid in the RAR.

Soil degradation

Study 1 ─ Phenmedipham

RAR B.8.1.1.1.1/02 (1976)

The degradation of [amino phenol-UL-^{14}C] and [methyl aniline-UL-^{14}C]phenmedipham was studied in two soil soils, loamy sand and sandy loam, for 224 days with a field application rate of 5 mg a.s./kg soil. The test systems were incubated in the dark for 60 days at 24°C and in soil moisture of 85 % and 58 % of water holding capacity (WHC) in loamy sand and sandy loam, respectively.

Mineralisation to CO_2 was 12.1 % and 25.5 % after 224 days in loamy sand and sandy loam soils, respectively. The kinetic evaluation was later performed according to FOCUS DegKinetics (RAR B.8.1.1.1.1/01, 2014). The non-normalised best fit (SFO) geomean DT_{50} values for [amino phenol-UL-^{14}C] and [methyl aniline-UL-^{14}C]phenmedipham were 95.8 and 22.3 days for loamy sand and sandy loam soils,

respectively. SFO DT_{50} values for degradate MHPC were 15.2 and 5.5 days for loamy sand and sandy loam soils, respectively.

The study was performed in 1976 and had no GLP compliance. It was performed in line with the OECD test guideline 307 except that the microbial activity measurements are missing. The Co-RMS considered this study not valid, however this study was considered valid in the RAR so it has been included in this classification proposal.

Study 2 ─ Phenmedipham

RAR B.8.1.1.1.1/03 (1991) & RAR B.8.1.1.1.1/04 (1991)

The degradation of [amino phenol-UL-^{14}C]phenmedipham was studied in the dark in German sandy loam soil in two different conditions: in a low moisture content of 24 % WHC and ambient temperature 21°C RAR B.8.1.1.1.1/03 (1991) and at low temperature 11°C with the recommended moisture content of 40 % of maximum water holding capacity (MWHC) (RAR B.8.1.1.1.1/04**,** 1991). The test substance was applied at a rate of 0.22 mg a. i./ 100 g soil. This corresponds to a field application rate of 1.65 kg/ha. The experiments were continued for 120 days and soil samples were taken after 0, 3, 7, 14, 21, 35, 56, 91 and 120 days.

Mineralisation to CO_2 was 16.5 % and 13.5 % after 120 days in low moisture and low temperature conditions, respectively. The kinetic evaluation was later performed according to FOCUS DegKinetics (RAR B.8.1.1.1.1/01, 2014). The non-normalised DT_{50} (FOMC) values of 15.2 and 24.4 days were obtained in low moisture and low temperature conditions, respectively. No DT_{50} value for MHPC was obtained.

These studies had no GLP compliance, but the studies were performed in line with the OECD test guideline 307 and the material balance was within 90-110 % of the applied radioactivity at the end of both tests. These studies were considered valid in the RAR.

Study 3 ─ Phenmedipham

RAR B.8.1.1.1.1/05 (2012)

The degradation of [methyl aniline-UL-^{14}C] phenmedipham was studies in four soils according to the OECD test guideline 307. Test systems were incubated in the dark for 35 days (sandy loam) and 120 days (silt loam, loam and loamy sand) at 20.5°C and a soil moisture of 55±5 % MWHC and under continuous ventilation with moistened air in an air-conditioned room. The pH and the organic carbon content of the four studied soils were within the recommended range (pH: 5.5-7.2 and orgC: 1.3-2.1 %). Mean material balances were 95.2-96.9 in all soils.

Mineralisation to CO_2 was 17.0 % after 35 days in sandy loam soil and 19.3, 23.9 and 24.8 % after 120 days in silt loam, loam and loamy sand soils, respectively. The kinetic evaluation was later performed according to FOCUS DegKinetics in RAR B.8.1.1.1.1/01 (2014). The non-normalised best fit (SFO) DT_{50} values for phenmedipham were 4.0, 19.9 and 44.6 days in sandy loam, silt loam and loamy sand soils, respectively. DFOP DT_{50} value was 29.9 days in loam soil for phenmedipham. No major degradation products were detected, but minor degradates, m-toluidine and AMPM, were detected but their concentration was always below 5 % of AR.

This study was performed according to the OECD test guideline 307 and was considered valid in the RAR.

Study 4 ─ Phenmedipham

RAR B.8.1.1.1.1/06 (1998)

The degradation of [amino phenol-UL-^{14}C]phenmedipham was studied in three soils with a field application rate of 1.45 kg per hectare. The test systems were incubated in the dark for 120 days (sand and loamy sand soils) and 99 days (sandy loam soils) at 20±2°C and soil moisture of 40-50 % MWHC and under continuous ventilation with moistened air in an air-conditioned room. The pH and the organic carbon content of the

studied three soils were within the recommended range of the OECD test guideline (pH 6.0-6.6 and orgC 0.59-2.27 %) and mean material balancies were between 92.2-95.1 % in all soils.

Mineralisation to CO_2 was 6.4 % after 99 days for sandy loam soil and 11.5 and 9.7 % after 120 days for sand and loamy sand soils, respectively. The kinetic evaluation was later performed according to FOCUS DegKinetics (RAR B.8.1.1.1.1/01, 2014). The non-normalised best fit (SFO) DT_{50} values were 22.6, 45.6 and 53.2 days for sandy loam, loamy sand and sand soils, respectively. No degradation products were characterised in the study. The total non-extractable residues in soil were 71.3 % and 71.5 % AR after 120 days in sand and loamy sand soil, respectively, and 73.8 % AR after 99 days in sandy loam soil.

This study was not performed in compliance with GLP, but it was performed in line with the OECD test guideline 307. This study was considered valid in the RAR.

Study 5 ─ *Phenmedipham*

RAR B.8.1.1.1.1/07 (1991)

The degradation of [amino phenol-UL-^{14}C]phenmedipham was studied in loamy sand soil with a field application rate of 1.65 kg per hectare. The test systems were incubated in the dark for 60 days at 22±2°C and soil moisture of 70 % at 1/3 bar water capacity and under continuous ventilation with moistened air in an air-conditioned room. The pH and the organic carbon content of the studied soil were within the recommended range of the OECD test guideline (pH 7.1 and orgC 2.45 %). Mean material balance was acceptable during the whole study period.

Mineralisation to CO_2 was 14.4 % after 60 days in loamy sand soil. The kinetic evaluation was later performed according to FOCUS DegKinetics (RAR B.8.1.1.1.1/01, 2014). The non-normalised best fit (SFO) DT_{50} value for phenmedipham was 13.5 days. No DT_{50} value for MHPC could be determined due to bad statistical and visual fit.

This study was performed in line with the OECD test guideline 307 and it was considered valid in the RAR.

11.1.4.4 Photochemical degradation

Phenmedipham was photochemically stable in the available studies (RAR B.8.2.1.2/01, 1992) & (RAR B.8.2.1.2/02, 2013) so direct photodegradation in water may contribute to a very limited extend to the degradation of phenmedipham in the environment.

Study 1 ─ *Phenmedipham*

RAR B.8.2.1.2/01 (1992)

The direct photolysis of phenmedipham was investigated in a sterile aqueous buffer at pH 4 following application of unlabelled phenmedipham with artificial sunlight (xenon light, 290 nm cut off) at 23°C. Sterile buffer solutions (pH 4; 1 % methanol) were treated with phenmedipham (4 mg/L) and the samples were exposed in 'merry-go-round' photoreactor for 17.7 days which corresponds to 35.4 days in natural sunlight. This study followed the OECD test guideline 316.

There were no differences between the amount of phenmedipham in irradiated and dark samples showing that phenmedipham is photolytically stable. This study is considered valid in the RAR.

Study 2 ─ Phenmedipham

RAR B.8.2.1.2/02 (2013)

The quantum yield of phenmedipham was solely determined in acidic buffer solution (pH 4/acetonitrile 9/1, v/v) at the concentration of 8.0 mg/L, because phenmedipham is not hydrolytically stable under neutral and alkaline conditions. The test system for the photodegradation experiment consisted of a merry-go-round irradiation apparatus at the temperature of 25±1°C. The quantum yield was calculated on the basis of UV absorption data and the degradation kinetics. This study followed the OECD test guideline 316.

The degradation of phenmedipham in a range of 3.5-4.8% was observed by HPLC/radiodetection after a maximum irradiation period of 500 min. The study results confirmed moderate degradability for direct photodegradation of phenmedipham with a mean quantum yield of $\Phi = 0.000136$, translating into estimated photolytic half-lives of about 36 to 77 days under environmental conditions.

This study is considered valid in the RAR.

11.1.5 Conclusion on rapid degradability

Phenmedipham was not readily biodegradable (RAR B.8.2.2.1/01, 1989) & (RAR B.8.2.2.1/03, 1985). Ready biodegradation of phenmedipham was 34.1 % (ThOD) and 54.3 % (DOC) after 14 days in RAR B.8.2.2.1/01 (1989) and 23-31 % (ThOD) after 35 days in RAR B.8.2.2.1/03 (1985). Inherent bioegradation of phenmedipham was 39.5 % (BOD/ThOD) and 19.2 % (DOC) after 28 days (RAR B.8.2.2.1/02, 1990).

Hydrolytic degradation of phenmedipham was strongly pH dependent. Phenmedipham undergoes rapid hydrolysis with the half-lives of few minutes in alkane (pH > 9) and few hours and days in neutral conditions and it was hydrolytically stable in the acidic conditions (RAR B.8.2.1.1/03, 2003), (RAR B.8.2.1.1/04, 2004) & (RAR B.8.2.1.1/05, 2015).

Half-lives in surface water for phenmedipham were 0.01-0.04 days (RAR B.8.2.2.2/01, 2013). Mineralisation to CO_2 reached its maximum of 15 % AR after 63 days for [methyl aniline-UL-^{14}C]phenmedipham, thus it cannot be demonstrated that phenmedipham was ultimately degraded, and the half-lives mainly represent primary degradation of phenmedipham. Degradation of phenmedipham in sterile systems supports the conclusion that phenmedipham was rapidly hydrolysed abiotically under the test condition.

Aquatic sediment simulation studies showed rapid dissipation and the maximum mineralisation to CO_2 was 54 % AR after 98 days (RAR B.8.2.2.3/05, 2015). Half-lives in soil for phenmedipham ranged from 4 to 53.2 days with the maximum mineralisation to CO_2 25.5 % AR after 224 days (RAR B.8.1.1.1.1/02, 1976).

Phenmedipham was photochemically stable in aquatic environment (RAR B.8.2.1.2/01, 1992) & (RAR B.8.2.1.2/02, 2013).

Consequently, phenmedipham is considered to be **not rapidly degradable** because:

- it was not readily biodegradable in ready biodegradation tests.
- hydrolytical degradation half-lives were not under 16 days in the whole pH range of 4.0-9.0 and the hydrolysis product m-toluidine fulfils the classification criteria as hazardous for the aquatic environment.
- it was not demonstrated that phenmedipham is ultimately degraded >70 % within 28 days in the aquatic environment and the degradation product m-toluidine fulfils the classification criteria as hazardous for the aquatic environment. In the surface water simulation test primary degradation was fast but the mineralisation to CO_2 was at maximum 15 % AR.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for the classification proposal of phenmedipham.

11.2.1 Summary of data/information on environmental transformation

11.3 Environmental fate and other relevant information

Study 1 ─ Phenmedipham

RAR B.8.1.3.1.1/06 (2010)

The adsorption of [methyl aniline-UL-^{14}C]phenmedipham to soil was investigated in 5 soils under standard conditions of batch equilibrium tests for 24 h for soils Wurmwiese, an Laacher Hof AXXa and Hanscheider

Hof and 4 h for soil Höfchen am Hohenseh at 20±2°C. This study was performed in line with OECD test guideline 106.

Phenmedipham was stable in the soils. The average amount of phenmedipham detected after 96 h was 94.3% AR. The adsorption behaviour of [methyl aniline-UL-^{14}C]phenmedipham was accurately described by the Freundlich equation within a nominal concentration range of 0.01 mg/L to 1.00 mg/L. The mean adsorption constants $K_{F\ (ads)}$ of the Freundlich isotherms ranged from 22.2 to 47.6. When being normalized for organic carbon content of soil, mean values of $K_{OC(ads)}$ ranged from 918 to 1618.

Table 36: Soil adsorption data of phenmedipham

Component / Soil	Texture	pH	OC	K_f	1/n	K_{oc}
	(USDA)		[%]	[mL/g]		[mL/g]
Phenmedipham						
Wurmwiese	Loam	5.3	1.8	24.2	0.79	1376
Höfchen am Hohenseh	Silt loam	6.6	2.4	22.2	0.80	918
Laacher Hof AXXa	Sandy loam	6.2	1.8	29.8	0.77	1618
Hanscheider Hof	Loam	5.6	3.1	47.6	0.79	1535
			Median	**24.2**	**0.79**	**1456**
			Arithmetic mean	**31.0**	**0.79**	**1362**
			Geometric mean	**29.5**	**0.79**	**1331**

This study is considered valid in the RAR.

11.4 Bioaccumulation

Summary of relevant studies on bioaccumulation from the Renewal Assesment Report is reported below. Only relevant and valid studies for the proposed classification of phenmedipham has been included from the RAR (draft RAR annexed to this CLH proposal).

Table 37: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Bioaccumulation: -OECD 305 GLP compliant	**Phenmedipham (0.02 mg/L)** **BCF** edibles 185 non-edibles 395 **whole fish 321** **Phenmedipham (0.2 mg/L)** **BCF** edibles 78 non-edibles 157 **whole fish 121**	Phenmedipham was almost totally hydrolysed in the study. Thus, the bioaccumulation potential mainly represent the degradation product MHPC. Test species used in this test was rainbow trout (*Oncorhynchus mykiss*).	1990 RAR B.9.2.2.3/01 M-146472-01-1
Bioaccumulation: -OECD 305 GLP compliant	**Phenmedipham (0.03 mg/L)** **BCF** viscera 1520 flesh 14 carcass 43 **whole fish 165**	Test species used in this test was bluegill sunfish (*Lepomis macrochirus*). **This is a key study.**	1988 RAR B.9.2.2.3/02 M-145979-01-1
Partition coefficient n-octanol/water: -OECD 117 -OCSPP 830.7570	**n-Octanol/water partition coefficient of phenmedipham** pH = 4 (20±1°C)	Purity 99.0 % (w/w). The partition coefficient was not determined in distilled water but in buffer pH 4.0 due to the	2012 RAR B.2.7/01 M-439458-01-1

Method	Results	Remarks	Reference
(HPLC method)	$logP_{o/w}$ = 2.7	lack of stability of the test item at pH values > pH 4.	
Partition coefficient n-octanol/water: -OECD 117 -OCSPP 830.7570 (HPLC method)	**n-Octanol/water partition coefficient of m-toluidine** pH = 5, 7, 9 (25°C) $logP_{o/w}$ = 1.6	Purity 98.5 % (w/w). No validation data has been presented.	2014 RAR B.2.7/01 M-485128-01-1
Partition coefficient n-octanol/water: -OECD 107 -OPPTS 830.7550 (Shake Flask method)	**n-Octanol/water partition coefficient of MHPC** pH = 5 (23°C): log $P_{o/w}$ = 1.2 pH = 7 (23°C): log $P_{o/w}$ = 1.3 pH = 9 (23°C): log $P_{o/w}$ = 1.2	Purity 98.5 % (w/w). Validation data has been presented.	2012 RAR B.2.7/01 M-427378-01-1

11.4.1 Estimated bioaccumulation

11.4.2 Measured partition coefficient and bioaccumulation test data

Study 1 ─ Phenmedipham

RAR B.9.2.2.3/01 (1990)

The juvenile fish (*Oncorhynchus mykiss*) were exposed in a flow-through system (flow rate 300 L/d) to a nominal concentration of [amino phenol -UL-14C]phenmedipham (purity 97.7 % w/w) of 0.02 mg/1 (115 fish) and 0.2 mg/L (106 fish) for 64 hours. Thereafter, the fish were transferred to flowing, untreated water for 128 hours and the elimination of radioactivity was followed. Ten fish were sampled at each sampling time. The mean body weight of the juvenile fish was 1.4-1.7 g.

Temperature ranged 15-16°C, pH 7.6-8.1 and oxygen 8.4-9.5 mg O_2/L. The residues were calculated as parent equivalents according to the radioactivity found in fish or fish parts, and are based on the fresh weight of the respective material.

The concentrations of the test solutions as parent equivalents were 0.017-0.021 mg/l and 0.180-0.191 mg/l (85-105 % and 90-96 % of the nominal), respectively. Chemical analysis showed that >86 % of the radioactivity was present as MHPC, 6.1-7.3 % as the parent phenmedipham and 5 % as an unknown metabolite in the higher test concentration after 21 hours and 68 hours.

The radioactive residues were rapidly accumulated during the uptake phase and achieved steady state concentrations within 11 hours (low dose) and 21 hours (high dose) of initial exposure. Steady state tissue concentrations in the low dose group were 3.517±0.151 ppm in edibles, 7.484±1.307 ppm in non-edibles and 6.097±0.806 ppm in whole fish. The corresponding values for the high target dose levels were 14.312±4.288 ppm, 28.965±3.336 ppm and 22.347±3.403 ppm, respectively.

The calculated BCF values are presented below.

Table 38: Bioconcentration potential of phenmedipham in fish (Oncorhynchus mykiss) (based on total 14C in fish and in water)

Test concentration	0.02 mg/l		0.2 mg/l	

	BCF	Depuration t½ (h)	BCF	Depuration t½ (h)
edibles	185	16.9	78	16.7
non-edibles	395	16.6	157	17.2
whole fish	321	16.6	121	17.2

This study is considered valid in the RAR. However RMS mentioned that phenmedipham was almost totally hydrolysed in the study and therefore the results mainly represent the bioaccumulation potential of the degradation product MHPC. It is noted that this test was performed before the introduction of lipid normalisation and growth-dilution correction to the OECD TG 305 so bioconcentration is based on steady state BCF value. Therefore, the BCF is not lipid normalised or corrected for fish growth and thus BCF could be higher or lower depending on the test fish lipid content and/or fish growth during the test period.

Study 2 ─ Phenmedipham

RAR B.9.2.2.3/02 (1988)

The juvenile fish (*Lepomis macrochirus*) were exposed in a flow-through system to methyl-phenol ring and phenyl ring labelled phenmedipham with nominal concentration of 0.03 mg/L for 10 days. The fish were then transferred into clean water and the depuration was followed for 6 days. The study conditions were temperature 22±1°C, pH 6.4-7.0, dissolved oxygen >8.0 mg/L and 12-hours light daily. The mean body length of the juvenile fish were 2.66 cm and the mean body weight 0.42 g at the beginning of the experiment. The mean body length of the juvenile fish were 3.32 cm and the mean body weight 0.84 g at the end of the experiment. The test solutions were maintained at low pH to prevent hydrolysis occurring.

Radiochemical analysis of the water was carried out daily during the exposure period and the water was analysed for the test compound, using HPLC technique. Concentration of ^{14}C-phenmedipham in fish during the exposure and the depuration was determined by ^{14}C analysis. Total ^{14}C residues in viscera, edible and non-edible tissues were analysed at predetermined intervals during the exposure and depuration phases. Five fish were used in each sampling time.

The mean measured concentrations for ^{14}C-phenmedipham equivalents determined in the exposure vessel with liquid scintillation counting (LSC) were 0.0257±0.0017 mg/L and 0.0313±0.0008 mg/L for themethyl-phenol and phenyl ring labels, respectively. The mean measured concentration determined by HPLC was 0.0185±0.0037 mg/L and 0.0238±0.0032 mg/L for the methyl-phenol and phenyl ring labels, respectively. Therefore, some hydrolysis occurred during the exposure.

The plateau concentration in fish was reached during the first day of the exposure. The mean concentration of total ^{14}C-residues in the fish during the plateau was 4.64 mg phenmedipham equivalent/kg. The mean BCF based on total ^{14}C residues in the viscera was 1520, in the flesh 14 and in the carcass 43, corresponding to a BCF value of 165 in the whole fish compared to the mean measured concentration of total ^{14}C-residues in water. There was no significant difference between the bioaccumulation of the two differently labelled test substances. During the 6 day depuration period the levels of ^{14}C-phenmedipham in the whole fish decreased approximately 70 % within 24 hours and 91 % within six days (the mean for the two label positions).

This study is considered valid in the RAR according to the validity criteria of the OECD test guideline 305. It is noted that this test was performed before the introduction of lipid normalisation and growth-dilution correction to the OECD TG 305 so bioconcentration is based on steady state BCF value. Therefore, the BCF is not lipid normalised or corrected for fish growth and thus BCF could be higher or lower depending on the test fish lipid content and/or fish growth during the test period.

11.5 Acute aquatic hazard

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

More details can be found in the annexed RAR.RARTable 39: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
Acute toxicity to fish - Phenmedipham					
OECD TG 203; US EPA OPPTS 835.1075 Semi-static 96 h GLP compliant	*Oncorhynchus mykiss* (rainbow trout)	Phenmedipham technical (purity 97.7 % w/w)	**LC_{50}:** 1.84 mg/L (mm)[1]	The NOEC is 0.117 mg a.s/L.	2016 RAR B.9.2.1/XX M-564852-01-1
Acute toxicity to fish - Degradate MHPC					
OECD TG 203 Static-renewal (daily) 96 h GLP compliant	*Oncorhynchus mykiss* (rainbow trout)	MHPC (methyl-(3-hydroxyphenyl)-carbamite) technical (purity 93.4 % w/w)	**LC_{50}:** 75 mg/L (mm)[1]	The NOEC was 32 mg/L based on the absence of any sub-lethal effects seen and no mortalities in the main test.	2000 RAR B.9.2.1/07 M-197965-01-1
OECD TG 203 Static 96 h GLP compliant	*Cyprinus carpio* (common carp)	MHPC (methyl-(3-hydroxyphenyl)-carbamite) technical (purity > 98 % w/w)	**LC_{50}:** > 100 mg/L (nom)[3]	No mortality occurred during the exposure period in the control or in the test concentration.	1998 RAR B.9.2.1/08 M-493442-01-1
OECD TG 203; US EPA OPPTS 835.1075; US EPA-FIFRA OPP 72–1 Static 96 h GLP compliant	*Pimephales promelas* (fathead minnow)	MHPC (methyl-(3-hydroxyphenyl)-carbamite) technical (purity 98.5 % w/w)	**LC_{50}:** > 100 mg/L (nom)[3]	The NOEC was empirically estimated to be 18.0 mg p.m./L. p.m. = pure metabolite	2013 RAR B.9.2.1/09 M-446967-01-1
Acute toxicity to fish – degrade m-toluidine					
Guideline not specified Static-renewal (twice a day) 96 h Non GLP	*Cyprinus carpio* (common carp)	m-toluidine technical (purity unknown)	**LC_{50}:** 93.3 mg/L (nom)[3]	There is no information whether the study followed any test guidelines.	2005 RAR B.9.2.1/10 M-462145-01-1

Acute toxicity to *Daphnia magna* - Phenmedipham					
OECD 202; JMAFF 12 Nounan No. 8147 Semi-static 48 h GLP compliant	*Daphnia magna* (cladoceran)	Phenmedipham (technical) Purity 97.4 % (w/w)	**EC_{50}:** 2.033 mg/L (mm)[1]	Study was conducted at the pH 6.0-6.5 due to rapid hydrolysis of phenmedipham at alkaline conditions.	2004 B.9.2.4.1/05 M-233654-01-1
Acute toxicity to *Daphnia magna* - Degradate MHPC					
OECD 202 Static 48 h GLP compliant	*Daphnia magna* (cladoceran)	MHPC (methyl-3-hydroxyphenylcarbamite) Purity 93.4 % (w/w)	**EC_{50}:** 14 mg/L (nom)[3]		2000 B.9.2.4.1/06 M-197966-01
OECD 202; ISO International standard 6341, EEC 92/69, Part C.2 Static 48 h GLP compliant	*Daphnia magna* (cladoceran)	MHPC (methyl-3-hydroxyphenylcarbamite) Purity 98 % (w/w)	**EC_{50}:** 26.6 mg/L (mm)[1]		1998 B.9.2.4.1/07 M-493443-01-1
Acute toxicity to *Daphnia magna* – Degradate m-toluidine					
OECD 202; USEPA OPPTS 850.1010; JMAFF 12 Nousan No. 8147 Static 48 h GLP compliant	*Daphnia magna* (cladoceran)	m-toluidine Purity 99.1 % (w/w)	**EC_{50}:** 0.1 mg/L (mm)[1]		2014 B.9.2.4.1/08 M-501339-01
Acute toxicity to *Americamysis bahia* – Phenmedipham					
OPPTS 850.1035 Flow-through 96 h GLP compliant	*Americamysis bahia* (mysid shrimp)	Phenmedipham (technical) Purity 99.1 % (w/w)	**EC_{50}:** 0.23 mg/L (mm)[1]	Periodic analyses of saltwater for potential contaminants were not conducted in accordance with GLP. However, these analyses were performed using a certified	2010 B.9.2.4.2/01 M-409871-01

				laboratory and standard U.S.EPA analytical methods.	
Acute toxicity to green algae – Degradate MHPC					
OECD 201 Static 96 h GLP compliant	*Pseudokirchneriella subcapitata* (green algae) (formerly known as *Selenastrum capricornutum*)	MHPC (methyl-3-hydroxyphenylcarbamite) Purity 93.4 % (w/w)	**72 h EC_{50} (biomass):** 30 mg/L (nom)[3] **96 h EC_{50} (biomass):** 26 mg/ (nom)[3] **72 h EC_{50} (growth rate):** 68.7 mg/L (nom)[3] **96 h EC_{50} (growth rate):** 79 mg/L (nom)[3]	The actual temperature recordings were not reported and the pH of the test medium increased more than 1.5 units required in OECD TG 201.	2000 RAR B.9.2.6.1/06 M-197967-01-1
OECD 201 Static 72 h GLP compliant	*Selenastrum capricornutum* (green algae)	MHPC (methyl-3-hydroxyphenylcarbamite) Purity >98 % (w/w)	**72 h EC_{50} (biomass):** 46 mg/L (nom)[3] **72 h EC_{50} (growth rate):** 240 mg/L (nom)[3]		1998 RAR B.9.2.6.1/07 M-493444-01-1
Acute toxicity to aquatic macrophytes – Phenmedipham					
ASTM guideline E 1415-91 (1991) Semi-static 14 d GLP compliant	*Lemna minor* (duck weed)	Phenmedipham (technical) Purity 99.4 % (w/w)	**7 d EC_{50} (biomass):** 0.109 mg/L (geo)[4] **7 d EC_{50} (growth rate):** >0.157 mg/L (geo)[4](geo)[4]	pH of the test medium increased more than 1.5 units required in OECD TG 201. Geometric mean concentrations were only calculated for 7 d endpoints	2004 RAR B.9.2.7/02 M-493457-01-1
OECD 239 Semi-static 14 d GLP compliant	*Myriophyllum spicatum* (Eurasian watermilfoil)	Phenmedipham (technical) Purity 97.7 % (w/w)	**EC_{50} (biomass):** 0.0519 mg/L (geo)[4] **EC_{50} (growth rate):** 0.0705 mg/L (geo)[4]	The pH of the test solution was purposely decreased to lower level than recommend in the TG 239 in order to prevent the hydrolysis as much as possible.	2017 RAR B.9.2.7/06 M-580251-02-1 **Key study**
Acute toxicity to aquatic macrophytes – Degradate MHPC					
OECD 221 Static 7 day GLP compliant	*Lemna gibba* (duck weed)	MHPC (methyl-3-hydroxyphenylcarbamite) Purity 97.5 % (w/w)	**EC_{50} (growth rate):** 26.8-27.3 mg/L (nom)[3]		2013 RAR B.9.2.7/03 M-451664-02-1
Acute toxicity to aquatic macrophytes – Degradate m-toluidine					
OECD 221 Semi-static 7 day GLP	*Lemna gibba* (duck weed)	m-toluidine Purity 99.1 % (w/w)	**EC_{50} (growth rate):** 92.2 mg/L (nom)[3] **EC_{50} (growth rate):** >100 mg/L (nom)[3]		2015 RAR B.9.2.7/04 M-512630-

compliant					01-1

[1] mm = mean measured concentration
[2] twa = time-weighted mean measured concentration
[3]nom = nominal concentrations
[4]geo = geometric mean concentrations
* According to the RAR, the study was conducted generally in line with the test method.

11.5.1 Acute (short-term) toxicity to fish

One acute toxicity test with phenmedipham, three with degradate MHPC and one with degradate m-toluidine on different fish species were considered valid in the RAR. The studies followed (with one exception) in general the OECD test guideline 203 "Fish, acute toxicity test" (1992). For phenmedipham, an experimental LC_{50} value of 1.84 mg/L was determined on *Oncorhynchus mykiss* (rainbow trout). For the degradate MHPC, experimental LC_{50} values of ≥ 75 mg/L were determined on *Oncorhynchus mykiss*, *Cyprinus carpio* (common carp) and *Pimephales promelas* (fathead minnow). For degradate m-toluidine, an experimental LC_{50} value of 93.3 mg/L was determined on *Cyprinus carpio*.

According to the CLP guidance, tests consistent with OECD test guideline 203 (or equivalent) should be used for classification. The endpoints are presented in table (Table 39) above and the studies are summarized below.

Study 1 – Phenmedipham

RAR B.9.2.1/XX (2016). Phenmedipham tech. (BCS-AD17874): Acute toxicity to rainbow trout (Oncorhynchus mykiss) under semi-static conditions.

Acute toxicity of phenmedipham to *Oncorhynchus mykiss* (rainbow trout) was studied (B.9.2.1/XX, 2016) in 96-hour test conducted according to guidelines OECD TG 203 (1992) and US EPA OPPTS 835.1075 (1996) and in compliance with GLP. Ten fish in each group, were exposed to water control, solvent control and nominal concentrations of 0.128, 0.282, 0.620, 1.36 and 3.00 mg a.s./L, corresponding to the geometric mean measured concentrations of 0.117, 0.250, 0.600, 1.24 and 2.73 mg a.s./L. During the semi-static test fish were observed after 0-4, 24, 48, 72 and 96 hours for behavioural responses, toxicity symptoms and mortality. The mortality (%) was calculated after 24, 48, 72 and 96 hours of exposure in each treatment group.

Before the test item preparation in all test aquaria was adjusted to a pH of approximately 6.5. This pH adaptation was done to stabilize the test item which is known to be more stable in acidic conditions. pH adaptation was performed also during the test. Dissolved oxygen concentrations ranged from 83 to 101 % oxygen saturation, the pH values ranged from 6.4 to 7.0 and the water temperature ranged from to 13.0 to 13.9 °C in all aquaria over the whole testing period. The photoperiod was 16 hours of light and 8 hours dark. In all groups, the concentrations of the test substance were measured at start of the test and on test solution renewals every 24 hours. The chemical analysis of phenmedipham (in water by HPLC – MS/MS) resulted in recoveries of 94-112 % of nominal at test initiation in the freshly prepared test media and the old test media recoveries ranged between 74 and 114 % of nominal. Therefore, the biological results are based on geometric mean measured concentrations of phenmedipham.

Validity criteria of the OECD test guideline 203 study were met (mortality in controls ≤ 10 % and dissolved oxygen > 60 %). The 96 hour LC_{50} value of 1.84 mg a.s./L was based on geometric mean measured concentration of phenmedipham. The NOEC for no effects is 0.117 mg a.s/L and the NOEC for adverse effects is 0.250 mg a.s./L.

Study 2 – degradate MHPC

RAR B.9.2.1/07 (2000). Acute toxicity to rainbow trout (Oncorhynchus mykiss) MHPC (methyl-3-hydroxyphenylcarbamate) Code: AE B038210.

Acute toxicity of degradate MHPC to *Oncorhynchus mykiss* (rainbow trout) was studied (B.9.2.1/07, 2000) in 96-hour test conducted according to OECD test guideline 203 (1992) and in compliance with GLP. The fish were exposed, in groups of 10, to an aqueous solution of the test material over a range of concentrations of 5.6, 10, 18, 32, 56 and 100 mg p.m./L for a period of 96 hours under semi-static test conditions. The number of mortalities and any sub-lethal effects of exposure in each test and control vessel were determined 3 and 6 hours after the start of exposure and then daily throughout the study until termination after 96 hours.

Dechlorinated laboratory tap water with a total hardness of approximately 100 mg/L as $CaCO_3$ was used. The water temperature was controlled at 14.0 °C with a mean dissolved oxygen content of 9.4 mg O_2/l and pH of 7.9 ± 0.1. These parameters were recorded daily. Fish were exposed in a photoperiod of 16 hours light and 8 hours darkness. A semi-static test regime was employed in the study involving a daily renewal of the test preparations. The concentration and stability of the test material in the test solutions were verified by chemical analysis at 0 (fresh media), 24 and 96 (old media) hours.

Validity criteria of the OECD test guideline 203 study were met (mortality in controls ≤ 10%, dissolved oxygen ≥ 80% and measured test concentrations ≥ 80 % of nominal concentrations). The 96 hours LC_{50} was calculated to be 75 mg p.m./L by the geometric mean method. The NOEC was 32 mg/L based on the absence of any sub-lethal effects seen and no mortalities in the main test.

Study 3 – degradate MHPC

RAR B.9.2.1/08 (1998). 96-Hour acute toxicity study in carp with methyl-(3hydroxyphenyl)-carbamate (static).

Acute toxicity of degradate MHPC to *Cyprinus carpio* (common carp) was studied (B.9.2.1/08, 1998) in 96-hour test conducted according to OECD test guideline 203 (1992) and in compliance with GLP. Seven fish per concentration were exposed in a limit test for 96 hours under static test conditions to a nominal concentration of 100 mg p.m./L against a control (dilution water). After 3, 24, 48, 72 and 96 hours of exposure the fish were inspected for the number of deaths, toxic symptoms or abnormalities. The mortality (%) after 3, 24, 48, 72 and 96 hours of exposure was calculated in each treatment group.

Within the study the pH value, the oxygen saturation level and the temperature were measured daily. Dissolved oxygen concentrations ranged from 7.0 to 9.2 mg/L, the pH values from 8.0 to 8.2 and the water temperature from 20.5 to 20.6 °C in all aquaria over the whole testing period. Hardness is 250 mg $CaC0_3$/L. The photoperiod was 16 hours of light and 8 hours dark.

Samples for analysis were taken at the beginning and in the end of the test from 100 mg p.m./L and the control and were analysed by HPLC - UV. The analytical determination of MHPC revealed recoveries of 101 % on day 0 and 103 % on day 4 of nominal test concentration of 100.0 mg p.m./L.

The endpoints were expressed in terms of nominal concentrations as they are in agreement with measured concentrations. Validity criteria of the OECD test guideline 203 study were met (mortality in controls ≤ 10 %, dissolved oxygen ≥ 80 % and measured test concentrations ≥ 80 % of nominal concentrations). The 96 hour LC50 was > 100 mg p.m./L.

Study 4 – degradate MHPC

RAR B.9.2.1/09 (2013). Acute toxicity of MHPC to the fathead minnow (Pimephales promelas) under static conditions.

Acute toxicity of degradate MHPC to *Pimephales promelas* (fathead minnow) was studied (B.9.2.1/09, 2013) in 96-hour test conducted in general according to guidelines OECD test guideline 203 (1992), US EPA OPPTS 835.1075 (1996) and US EPA-FIFRA OPP 72–1 (1982) and in compliance with GLP. Ten fish in each group, were exposed for 96 hours under static conditions to five concentrations of the test item. The following nominal (mean measured) concentrations were included in the study: control, 10.0 (10.1), 18.0 (17.8), 32.0 (33.4), 56.0 (56.0) and 100 (101) mg p.m./L. Mortalities among the test fish, and any observable abnormal behavioural responses, were recorded at 4, 24, 48, 72 and 96 hours.

At the beginning of the test, and every 24 hours thereafter, dissolved oxygen and pH of the control, and each test concentration were determined. The pH values ranged approximately from 7.4 to 8.1, the O_2-saturation ranged from 5.6 mg/L (67 % saturation) to 8.2 mg/L (98 % saturation) and the mean temperature was 24.3 °C. The photoperiod was 16 h light: 8 hours darkness.

Chemical analysis was performed on day 0 and day 4. Samples were analysed by HPLC with a LOQ of 1 mg/L. Mean measured recoveries ranged from 99 to 104 % of nominal values. Therefore, the results are based on nominal concentrations.

Only one fish died in 56 mg p.m./L concentration and no other mortalities occurred. The total length of the fish exceeded the recommended length of 2.0 ± 1.0 cm which is a small deviation from the guideline and not considered to have an impact on the results. Otherwise, the validity criteria (mortality in controls ≤ 10 %, dissolved oxygen ≥ 80 % and measured test concentrations ≥ 80 % of nominal concentrations) of the OECD TG 203 study were considered met. The 96 h LC_{50} for MHPC was determined to be > 100 mg p.m./L based on nominal concentrations. The NOEC was empirically estimated to be 18.0 mg p.m./L.

Study 5 – degradate m-toluidine

RAR B.9.2.1/10 (2005). Quantitative structure-activity relationships for the toxicity of substituted benzenes to Cyprinus carpio.

Acute toxicity of degradate m-toluidine to *Cyprinus carpio* (common carp) was studied (B.9.2.1/10, 2005) in a semi-static 96-hour test. There is no information whether the study followed any test guidelines. Two replicates of 10 fish were exposed to at least five different concentrations (with the same logarithmic difference in concentrations between substances). Temperature of the test water ranged from 15 to 18 °C, dissolved oxygen was 6.35 mg/L, and pH was 7.0-7.5. During the test, the half of the test water was replaced twice a day. The test compound concentrations were not analysed from the test medium.

No validity criteria (control mortality or test substance stability) were reported or discussed in the article. The experimental log ($1/LC_{50}$) for m-toluidine was 3.06 mol/L i.e. 93.3 mg/L.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

One acute toxicity study for water flea *Daphnia magna* and mysid *Americamysis bahia* were considered valid in the RAR for phenmedipham. The lowest toxicity was 96 h EC_{50} value of 0.23 mg/L for *Americamysis bahia* based on mean measured concentrations. For degradate MHPC two acute toxicity studies for *Daphnia magna* and for degradate m-toluidine one *Daphnia magna* study were considered valid in the RAR.

Study 1 – Phenmedipham

RAR B.9.2.4.1/05 (2004). Phenmedipham (technical): Acute immobilisation test with daphnids (*Daphnia magna*) under semi-static conditions.

Acute toxicity of phenmedipham (purity 97.4 % w/w) to *Daphnia magna* was studied in 48 h semi-static test according to OECD TG 202 and in compliance with GLP. *Daphnia magna* (<24 hours old) were exposed to nominal concentrations of 0.00625, 0.0625, 0.625, 1.25, 2.50, 5.00 and 10.0 mg/L at pH 6.0-6.5 in 4 replicates of 5 daphnids in each. In addition, a control (untreated daphnid medium) and a solvent control (containing 0.1 mL/ N,N-Dimethylformamide) were tested. The numbers of immobilised daphnids in each replicate test vessel and biological observations were recorded after 0, 12, 24, 36 and 48 hours of exposure. At hours 12, 24 and 36, the aged test solutions of the control, solvent control and the treated test solutions were replaced with the freshly prepared test solutions. Test solutions were sampled and measured at hours 0 and 24 for fresh media and 12 and 36 for aged test solutions for phenmedipham by HPLC. Temperature, dissolved oxygen concentration and pH were measured in the excess of the freshly prepared test solutions at hour 0, 12, 24 and 36. At hours 12, 24, 36 and 48, temperature, dissolved oxygen concentration and pH of

the aged test solutions were measured in combined samples. Temperature ranged from 20.2-21.1°C and oxygen concentrations from 7.10-8.94 mg/L.

Measured concentrations of phenmedipham were within 80 to 120 % of the nominal concentrations for fresh solutions except for the two highest treatment levels for which the concentrations were above the practical limit of solubility of the test item in dilution water. The solubility was determined to be 1.91 mg test item/L. Mean recoveries in aged solutions ranged from 55.3-90.1 % of nominal concentration. The study fulfils the validity criteria set in the OECD TG 202 and is considered valid in the RAR. The 48 h EC_{50} was determined to be 2.033 mg/L for phenmedipham based on mean measured concentrations.

Study 2 – Phenmedipham

RAR B.9.2.4.2/01 (2010). Phenmedipham: A 96-hour flow-through acute toxicity test with the saltwater mysid (*Americamysis bahia*).

Acute toxicity of phenmedipham (purity 99.1 % w/w) to *Americamysis basia* was studied in 96 h flow-through test according to OPTTS guideline 850.1035 and in compliance with GLP. Juvenile *Americamysis bahia* (20 per treatment level) were exposed to six nominal test concentrations (0.063, 0.13, 0.25, 0.50, 1.0 and 2.0 mg/L), a negative control (dilution water) and a solvent control (0.1 mL/L acidified dimethylformamide). Two replicate test chambers were maintained in each treatment and control group, with 10 saltwater mysids in each test chamber. Observations of mortality and other signs of toxicity were made approximately 5, 24, 48, 72 and 96 hours after test initiation. Cumulative percent mortality observed in the treatment groups was used to determine LC_{50} values at 24, 48, 72 and 96 hours. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, at approximately 3 hours after initiation, at 24, 48, 72 hours and at the end of the test by HPLC-UV. The LOQ for phenmedipham and MHPC were 0.00185 and 0.00279 mg/L, respectively. Temperature during the study was 25±2°C, pH 8.0-8.2 and dissolved oxygen 4.6-7.2 mg O_2/L. The photoperiod was 16 hours of light and 8 hours of dark.

The samples from highest treatment group were centrifuged due to observation of some precipitation in the water of test chambers. The mean measured test concentrations were 0.028, 0.10, 0.23, 0.48, 0.88, 2.0 (non-centrifuged) and 1.9 mg/L (centrifuged), representing 44, 77, 93, 96, 88, 100 (non-centrifuged) and 95 % (centrifuged) of nominal concentrations, respectively. The lower recoveries in the 0.063 mg/L treatment group were most likely due to testing at the limit of the analytical method.

The study fulfils the validity criteria set in the OPPTS guideline 850.1035 and is considered valid in the RAR. According to the OPPTS Guideline a range finding test should be performed to determine which life stage, juvenile or young adults, is to be utilized in the definitive test. This was not done, but RMS did not consider this to invalidate the study. The 96 h EC_{50} was determined to be 0.23 mg/L for phenmedipham based on mean measured concentrations.

Study 3 – degradate MHPC

RAR B.9.2.4.1/06 (2000). Acute toxicity to *Daphnia magna* Methyl-3-hydroxyphenylcarbamate (MHPC) substance technical 93 % w/w Code: AE B038210 00 1D93 00.

Acute toxicity of degradate MHPC (purity 93.4 % w/w) to *Daphnia magna* was studied in 48 h static test according to OECD TG 202 and in compliance with GLP. *Daphnia magna* (<24 hours old) were exposed to nominal concentrations of 1.0, 1.8, 3.2, 5.6, 10, 18, 32, 56 and 100 mg/L in 2 replicates of 10 daphnids in each. Immobilisation was recorded at 24 and 48 hours. The concentration of MHPC was verified by chemical analysis at 24 and 48 hours by HPLC-UV. The measured test concentrations ranged from 115-120 % of nominal with the exception of the 3.2 mg/L, which showed measured values of 125 and 124 % of nominal at 0 and 48 hours, respectively.

The study fulfils the validity criteria set in the OECD TG 202 and is considered valid in the RAR. The 48 h EC_{50} was determined to be 14 mg/L for MHPC based on nominal concentrations.

Study 4 – degradate MHPC

RAR B.9.2.4.1/07 (1998). Acute toxicity study in *Daphnia magna* with methyl-(3-hydroxyphenyl)-carbamate (static).

Acute toxicity of degradate MHPC (purity 98 % w/w) to *Daphnia magna* was studied in 48 h static test according to OECD TG 202 and in compliance with GLP. *Daphnia magna* 1st instars <24 hours old were exposed to nominal concentrations of 0, 10.0, 18.0, 32.0, 56.0 and 100.0 mg/L, corresponding to mean measured concentrations of 0, 10.2, 32.4 and 105 mg/L. After 24 and 48 hours the behaviour of the water fleas was visually evaluated by counting immobile daphnids. The concentration of MHPC in exposure media was measured from the test item concentrations 10, 32 and 100 mg/L at start and at the end of the exposure period by HPLC. Dissolved oxygen concentrations ranged from 8.2-8.3 mg O_2/L, the pH values from 8.6-9.6 and the temperature in the control from 20.3-20.4°C. The photoperiod was 16 hours of light and 8 hours dark.

The study fulfils the validity criteria set in the OECD TG 202 and is considered valid in the RAR. The 48 h EC_{50} was determined to be 25.6 mg/L for MHPC based on mean measured concentrations.

Study 5 – degradate m-toluidine

RAR B.9.2.4.1/08 (2014). Acute toxicity of BCS-AU61245 (m-toluidine) to the water flea *Daphnia magna* in a static laboratory test system - Final report.

Acute toxicity of degradate m-toluidine (purity 99.1 % w/w) to *Daphnia magna* was studied in 48 h static test according to OECD TG 202 and in compliance with GLP. *Daphnia magna* 1st instars <24 hours old were exposed to nominal concentrations of 0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/L, corresponding to mean measured test concentrations of 0.0175, 0.0342, 0.0684, 0.142, 0.295 and 0.582 mg/L. Immobility was assessed at 24 and 48 hours after starting the test. The concentration of m-toluidine in exposure media was measured in freshly prepared and in aged test media. The temperature ranged from 21.0–21.3°C and pH from 7.9-8.0. The photoperiod was 16 hours of light and 8 hours dark.

The chemical analysis of m-toluidine in the freshly prepared test solutions at test initiation revealed measured concentrations of 95.6-107.4 % of the nominal. The corresponding concentrations of the aged test solutions at the end of the 48 hours exposure period were 67.5-88.4 % of nominal.

The study fulfils the validity criteria set in the OECD TG 202 and is considered valid in the RAR. The 48 h EC_{50} was determined to be 0.1 mg/L for m-toluidine based on mean measured concentrations.

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

During the Peer Review of phenmedipham the validity of available algae studies were discussed, since in most cases the analytical results showed initial residues, but at next sampling times the residues were below the LOQ. The endpoints based on geomean concentration of phenmedipham used by RMS was not fully supported during the Peer Review Expert meeting 133. It is stated in the report of the meeting that studies where the geomean measured concentrations are calculated based on mean initial concentrations and LOQ/2 are not considered valid. Hence, studies with no intermediate samples with measurable residues were not considered valid then. Therefore, those studies which were not considered valid are not presented in this CLH-dossier either though can be found in the annexed RAR of phenmedipham.

No valid chronic toxicity data are available for algae, as reliable endpoints were not possible to derive due to rapid hydrolysis of the phenmedipham in available algal studies in the RAR. However, there are valid chronic toxicity studies available for aquatic macrophytes duck weed *Lemna minor* and Eurasian watermilfoil *Myriophyllum spicatum* for phenmedipham (RAR B.9.2.7/02, 2004 & RAR B.9.2.7/06, 2017). The lowest acute toxicity was a 14 day EC_{50} value of 0.0705 mg/L for *Myriophyllum spicatum* based on geometric mean measured concentrations (B.9.2.7/06). This is used as a key study for acute aquatic hazard classification of phenmedipham.

For degradate MHPC two valid algal and one duck weed study were available in the RAR and are summarised below. For degradate m-toluidine one valid duck weed study was available in the RAR and is also summarised in this chapter.

Study 1 – Phenmedipham

RAR B.9.2.7/02 (2004). Phenmedipham - Aquatic plant toxicity test, *Lemna minor*, semi-static, 14 d.

Toxicity of phenmedipham (purity 99.4 % w/w) to *Lemna minor* was studied in 14 d semi-static (renewal of test media every 2-3 d) aquatic plant toxicity test according to ASTM guideline E 1415-91 and in compliance with GLP. *Lemna minor* plants were exposed to initial mean measured concentrations of 1.76, 0.020, 0.048, 0.11, 0.28, 0.69 and 1.76 mg/L and control. The test system consisted of three replicate vessels per test concentration and a control. Frond numbers were determined at the start and at the end of the test and every renewal date of the test media. Therefore, frond numbers were assessed on days 0, 3, 5, 7, 10, 12 and 14 and inhibition of log biomass growth and specific growth rate were determined. The concentrations of phenmedipham and its degradate MHPC were analysed on day 0, 3 and 7 (freshly prepared solutions) and on days 3, 5 and 10 (old solutions) via HPLC-UV from all test levels. The LOQ was fixed to 0.01 mg/L for phenmedipham and MHPC. The pH values ranged from 6.33-8.12 (0-7 d), the temperature from 23-25°C and the light intensity from 5730-7240 Lux.

The initial concentration of phenmedipham were in the range of 83 to 107 % of nominal. RMS requested that the endpoints should be calculated based on geometric mean measured concentrations of phenmedipham.

Task Force of Phenmedipham provided following calculations:

Sampling time (d)	Measured concentrations (2 sampling intervals = first 5 days covered)					
Inl measured conc (mg/L)	0.02	0.048	0.11	0.28	0.69	1.76
0	0.021	0.05	0.12	0.3	0.74	1.91
3	0.01	0.005	0.005	0.005	0.005	0.014
3	0.021	0.048	0.11	0.28	0.7	1.82
5	0.012	0.015	0.005	0.005	0.005	0.012
geomean conc.	0.0150	0.0195	0.0241	0.0382	0.0602	0.1570

Highlighted cell: measured value below LOQ (0.01 mg/L) so half the LOQ is used for calculations

The measured concentrations were only available for the renewal intervals at day 0-3 and at day 3-5 covering the first five days of the study period of seven days. Thus, the calculation of geometric mean measured concentrations was based on 2 renewal intervals as no information is available on the interval days 5-7. Where measured concentrations at the renewal intervals were below LOQ, such concentrations are considered to be half of the LOQ.

Table 40: ECx values for study parameter frond number

Endpoint (0-7 day)	Growth rate		Yield	
	ini. measured conc.	geomean measured conc.	ini. measured conc.	geomean measured conc.
EC_{10} [mg/L] (95% confidence interval)	0.314 (0.183 - 0.437)	0.044 (0.032 - 0.054)	0.108 (0.055 - 0.166)	0.022 (0.016 - 0.028)
EC_{20} [mg/L] (95% confidence interval)	0.859 (0.660 - 1.101)	0.090 (0.075 - 0.107)	0.247 (0.160 - 0.333)	0.038 (0.031 - 0.046)
EC_{50} [mg/L] (95% confidence interval)	> 1.76 (n.d.)	> 0.157 (n.d.)	1.202 (0.928 - 1.696)	0.109 (0.091 - 0.138)
LOEC [mg/L]	0.280	0.038	0.280	0.038
NOEC [mg/L]	0.110	0.024	0.110	0.024

The 7 d EC_{50} value of >0.157 mg/L for growth rate and the 7 d EC_{50} value of 0.109 mg/L for biomass growth inhibition was determined based on geometric mean concentrations of phenmedipham. The study fulfilled the validity criteria set in OECD TG 221. The pH-value did increase by more than 1.5 units but this is not considered to invalidate the study since the validity criteria for control growth was met and thus RMS considered this study valid in the RAR.

Study 2 – Phenmedipham

RAR B.9.2.7/06 (2017). Toxicity of phenmedipham tech. (BCS-AD17874) to the Aquatic Plant *Myriophyllum spicatum* in a Semi-Static Growth Inhibition Test.

Toxicity of phenmedipham (purity 97.4 % w/w) to *Myriophyllum spicatum* was studied in 14 d semi-static aquatic plant toxicity test according to OECD TG 239 and in compliance with GLP. Shoots of *Myriophyllum spicatum* were exposed via the water phase under defined conditions in a water-sediment test system to control, solvent control (dimethylformamide, 0.1 mL) and nominal concentrations of 3.0, 9.49, 30.0, 94.9 and 300 µg/L of test item. Four replicates per test concentration and six replicates for each control was used. The corresponding geometric mean measured concentrations were 0.898, 3.85, 12.8, 46.4 and 170 µg/L. After an establishment phase of 7 days, 3 plants per replicate were exposed for 14 days under semi-static conditions. The test water and sediment was according to OECD TG 239, except for water pH to enhance the stability of the otherwise quickly hydrolysing test substance. The pH of the test media was lowered to 6.5 before preparation of the test concentrations. The photoperiod during the study was 16 h light and 8 h darkness at a mean light intensity of 144 $\mu E*m^{2}*s^{-1}$. The temperature ranged from 19.4-24.1°C, pH value in fresh test media from 6.5-6.7 and in the aged test media from 6.3-6.8, oxygen concentrations in the freshly prepared test media from 8.6-9.1 mg/L, and in the aged test media from 0.8-12.5 mg/L (less than 10 mg/L in two highest test concentrations in aged media). Concentrations of phenmedipham and its degradate MHPC were measured in all freshly prepared test levels on days 0, 4, 7 and 11 and additionally in all aged test levels on days 4, 7, 11, and 14 of the exposure period via HPLC-MS/MS method. LOQ was 0.2 µg/L.

The total shoot length was determined at the test start, on days 0, 7 and 14. On day 14, the fresh and dry weight of each plant were determined. The inhibition of growth in relation to control plants was determined over an exposure period of 14 days using two response variables, average specific growth rate and biomass (yield). Visual assessment of plant health was performed on every shoot length measurement occasion. Any sublethal symptoms, for example necrosis, chlorosis, stunting, altered internodial length or loss of turgor were observed for each plant. Additionally, all test beakers were checked for the development of bacterial, fungal or algal contamination. Visual assessment of root health was performed at test end by comparing the development of the root systems of each exposed plant to the control root systems.

In the freshly prepared test media 51-113 % of the nominal test concentration was found (average of all test concentrations). In the aged test media, 12-38 % of the nominal value was determined (average of all test concentrations) for all test concentrations except for 3.0 µg/L on Day 4 where recovery was <LOQ. Since the

phenmedipham concentrations decreased within the water exchange intervals, the geometric mean measured concentration was calculated for each treatment group and used for the endpoint calculations.

During the whole testing period, no sublethal effects were recorded in any test concentration. The visual assessment of the roots at the end of the test revealed no effect caused by the test substance. All roots were well developed.

Table 41: Inhibition of growth rates of *Myriophyllum spicatum*

Geometric mean concentration [µg/L]	Length Growth Rate (cm^{-1}) mean±SD	Inhibition of growth rate for total shoot length (%)	Wet Weight Growth Rate (g^{-1}) mean±SD	Inhibition of growth rate for total plant wet weight (%)	Dry Weight Growth Rate (g^{-1}) mean±SD	Inhibition of growth rate for total plant dry weight (%)
Pooled control	0.063±0.008	-	0.084±0.005	-	0.042±0.010	-
Solvent control	0.063±0.009	-	0.079±0.009	-	0.039±0.010	-
0.898	0.065±0.007	-3.9	0.083±0.018	-2.3	0.044±0.007	-7.4
3.85	0.071±0.008	-13.0	0.086±0.009	-5.3	0.043±0.017	-4.9
12.8	0.067±0.014	-6.7	0.079±0.009	3.3	0.033±0.009	19.8
46.4	0.056±0.009	11.3	0.068±0.013	17.1*	0.026±0.014	36.4*
170	0.040±0.009	36.1*	0.049±0.009	40.1*	0.022±0.007	70.4*

Based on growth rate, the most sensitive parameter was dry weight with the 14 d EC_{50} value of 0.0705 mg/L was determined based on geometric mean measured concentrations of phenmedipham. For biomass growth (dry weight of yield) The 14 d EC_{50} value of 0.0519 mg/L was determined based on geometric mean measured concentrations of phenmedipham.

The study was performed according to the OECD TG 239 and the test conditions were in line with the TG, except for the pH which was lower than recommended 7.9. The pH of the test solution was purposely decreased in order to prevent the hydrolysis of phenmedipham as much as possible. Phenmedipham is hydrolytically very unstable in alkaline medium with DT_{50} values of 3-24 hours in pH 7.0-7.5 and 3 hours in pH 8.0. This is not considered to have effect on the study results since the validity criteria were met. Hence, RMS considered this study valid in the RAR.

Study 3 – degradate MHPC

RAR B.9.2.6.1/06 (2000). Algal inhibition test - *Pseudokirchneriella subcapitata* MHPC (methyl-3-hydroxyphenylcarbamate) Code: AE B038210.

Toxicity of degradate MHPC (purity 93.4 % w/w) to *Pseudokirchneriella subcapitata* was studied in 96 h static algal growth inhibition test according to OECD TG 201 and in compliance with GLP. *Pseudokirchneriella subcapitata* were exposed to nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L in comparison to a control. Three replicates were assessed for each dilution level and for control group. The initial cell density was $1x10^4$ cells per mL. The pH values ranged from 7.6-9.9 at 96 hours and the incubation temperature was stated to be within 24±1°C. Samples for analysis of the test concentration were taken at the beginning and at the end of the test from three concentrations and analysed by HPLC. The measured test

concentrations at 0 and 96 hours ranged from 89-98 % of the nominal values and therefore the results are based on nominal test concentrations.

The 72 h EC_{50} value of 30 mg/L and a 96 h EC_{50} value of 26 mg/L based on biomass inhibition were determined for MHPC. The 72 h EC_{50} value of 68.7 mg/L and 96 h EC_{50} value of 79 mg/L based on growth rate inhibition were determined for MHPC.

The study fulfilled the validity criteria set in OECD TG 201 for control growth and the coefficient of variation for section-by-section specific growth rate and average specific growth rate during the whole test period in control. However, the study has some limitations, the actual temperature recordings were not reported and the pH of the test medium increased more than 1.5 units required in OECD TG 201. Since the validity criteria for control growth was reached the RMS considered this study valid in the RAR.

Study 4 – degradate MHPC

RAR B.9.2.6.1/07 (1998). Fresh water algal growth inhibition test with methyl-(3-hydroxyphenyl)-carbamate.

Toxicity of degradate MHPC (purity >98 % w/w) to *Selenastrum capricornutum* was studied in 72 h static algal growth inhibition test according to OECD TG 201 and in compliance with GLP. *Selenastrum capricornutum* were exposed in two tests to nominal concentrations of 0.1, 0.18, 0.32, 0.56 and 1.0 mg/L and 1.0, 2.2, 4.6, 10.0, 22.0, 46.0 and 100 mg/L in comparison to a control. The test system consisted of three replicate vessels per test level and six replicate vessels per control. The initial cell number was $1x10^4$ cells/mL. Growth inhibition was calculated using algae biomass per volume. The surrogate for biomass was cell density (used as response parameter) which was analysed spectrophotometrically. The pH values ranged from 8.0-8.3 in the two EC_{50} tests in the controls and the incubation temperature ranged from 21.3-22°C (first EC_{50} test) and 22.0-23.0°C (second EC_{50} test). Concentration of MHPC was measured by HPLC from three concentrations 0.10, 0.32 and 1.0 mg/L and the negative control in the first EC_{50} test and from 1.0, 10.0 and 100.0 mg/L and the negative control in the second EC_{50} test.

The concentrations of MHPC in the treatment levels found on day 0 were 101-108% of nominal in the first EC_{50} test and 87-97% of nominal in the second EC_{50} test. On day 3 concentrations of MHPC were 93-111 % of nominal in the first test and 90-102 % of nominal in the second test.

The 72 h EC_{50} for cell growth inhibition was 46 mg/L based on nominal concentrations of MHPC. The 72 h EC_{50} for growth rate reduction was estimated to be 240 mg/L based on nominal concentrations of MHPC. The study fulfilled the validity criteria set in OECD TG 201 and is considered valid in the RAR.

Study 5 – degradate MHPC

RAR B.9.2.7/03 (2013). Lemna gibba G3 - Growth inhibition test with BCS-AA66045 (phenmedipham-MHPC) under semi-static conditions - Amendment 1 to report.

Toxicity of degradate MHPC (purity 97.5 % w/w) to *Lemna gibba* was studied in 7 d static aquatic plant toxicity test according to OECD TG 221 and in compliance with GLP. *Lemna gibba* plants were exposed to the nominal concentrations of solvent control (Dimethylformamide), 1.13, 2.25, 4.50, 9.00 and 18.0 mg/L in comparison to a control. The test system consisted of three replicates of 12 fronds in each per test level and control. Visual observations were made on study days 2, 4, and 7. The pH values ranged from 7.5-9.0 and the temperature from 23.9-25.3°C over the whole period of testing. Concentration of MHPC was measured on day 0 and on day 7 of the exposure period in all test levels by HPLC-MS/MS.

The measured concentrations of MHPC in the treatment levels on day 0 ranged from 84-114% of nominal (average 96.2%). On day 7, measured concentrations ranged from 92-122% of nominal (average 103%). All reported results are based on nominal concentrations of the test item. No visual effects on *Lemna gibba* were observed. The 7 d EC_{50} values of 26.8 mg/L and 27.3 mg/L based on growth rate inhibition for frond number and total frond area were determined for MHPC, respectively. The study fulfilled the validity criteria set in the OECD TG 221 and is considered valid in the RAR.

Study 6 – degradate m-toluidine

RAR B.9.2.7/04 (2015). Lemna gibba G3 - Growth inhibition test with BCS-AA61245 (m-toluidine) under semi-static conditions – final report.

Toxicity of degradate m-toluidine (purity 99.1 % w/w) to Lemna gibba was studied in 7 d semi-static aquatic plant toxicity test according to OECD TG 221 and in compliance with GLP. Lemna gibba plants were exposed to the nominal concentrations of 0, 0.298, 0.954, 3.05, 9.77, 31.3 and 100 mg/L in comparison to a control. The test system consisted of four replicates of 12 fronds in each per test level and a control. Visual observations were made on study days 3, 5, and 7. The pH values ranged from 7.5-8.9 and the temperature from 24.4-24.9 °C over the whole period of testing. The test item was applied into the freshly prepared test medium on day 0, 3 and 5. The test concentrations were measured from fresh media on days 0, 3 and 5 and in old media on days 3, 5 and 7 by HPLC/UV/VIS-detection.

The concentrations of m-toluidine found in all freshly prepared test levels on day 0, 3, and 5 ranged between 90 and 100 % of nominal concentrations. In aged test levels on days 3, 5, and 7, concentrations ranged between 88 and 99 % of nominal. Therefore, all reported results are based on nominal concentrations. The 7 d EC_{50} values of >100 and 92.2 mg/L for the growth rate inhibition (frond number and total frond area) were determined for degradate m-toluidine, respectively. The study fulfilled the validity criteria set in the OECD TG 221 and is considered valid in the RAR.

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

11.6 Long-term aquatic hazard

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

RAR**Table 42: Summary of relevant information on chronic aquatic toxicity**

Method	Species	Test material	Results	Remarks	Reference
Chronic toxicity to fish- Phenmedipham					
OECD TG 210; US EPA OCSPP 850.1400; US EPA-FIFRA OPP 72–4 Flow-through 92 d (60 d post-hatch) GLP compliant	*Oncorhynchus mykiss* (rainbow trout)	Phenmedipham technical (purity 99.1 % w/w)	**NOEC(fry survival):** 0.096 mg/L (mm)[1] **NOEC(percent hatch):** 0.361 mg/L (mm)[1] **NOEC(percent swim-up):** 0.181 mg/L (mm)[1] **NOEC(standard length growth):** 0.041 mg/L (mm)[1] **NOEC(dry weight growth):** 0.096 mg/L (mm)[1] **NOEC(morphological and behavioural effect):** 0.041 mg/L (mm)[1]	The aforementioned guidelines were harmonized for various test parameters (i.e. temperature, light, etc.) to achieve optimal environmental conditions for the test organism. Scientific discretion was implemented where guideline parameters do not fully converge.	2014 RAR B.9.2.2.1/01 M-481742-01-1 **Key study**
Chronic toxicity to fish – Degradate MHPC					
OECD TG	*Oncorhynchus*	MHPC (methyl-	**NOEC(fry survival):** ≥ 10.4	The aforementioned	2014

210; US EPA OCSPP 850.1400; US EPA-FIFRA OPP 72–4 Flow-through 95 d (62 d post-hatch GLP compliant	*mykiss* (rainbow trout)	(3-hydroxyphenyl) -carbamite) (purity 97.5 % w/w)	mg/L (mm)[1] **NOEC(percent hatch):** ≥ 10.4 mg/L (mm)[1] **NOEC(percent swim-up):** ≥ 10.4 mg/L (mm)[1] **NOEC(standard length growth):** 5.34 mg/L (mm)[1] **NOEC(dry weight growth):** 2.74 mg/L (mm)[1] **NOEC(morphological and behavioural effect):** 5.34 mg/L (mm)[1]	guidelines were harmonized for various test parameters (i.e. temperature, light, etc.) to achieve optimal environmental conditions for the test organism. Scientific discretion was implemented where guideline parameters do not fully converge.	RAR B.9.2.2.1/02 M-482035-01-1
Chronic toxicity to *Daphnia magna* - Phenmedipham					
OECD 211; USEPA OCSPP 850.1300 Flow-through 21 d GLP compliant	*Daphnia magna* (cladoceran)	Phenmedipham (technical) Purity 99.1 % (w/w)	**NOEC(reproduction):** 0.005 mg/L (mm)[1] **NOEC(survival):** 0.026 mg/L (mm)[1]	RMS asked Task force Phenmedipham to recalculate endpoints based on arithmetic mean measured concentrations for phenmedipham	2014 RAR B.9.2.5.1/03 M-482048-01-1 **Key study**
Chronic toxicity to *Daphnia magna* – Degradate m-toluidine					
OECD 211; USEPA OCSPP 850.1300 Semi-static 21 d GLP compliant	*Daphnia magna* (cladoceran)	m-toluidine Purity 99.1 % (w/w)	**NOEC(reproduction):** 0.00467 mg/L (twa)[2] **EC_{10} (reproduction):** 0.00478 mg/L (twa)[2]		2015 RAR B.9.2.5.1/04 M-532928-01-1 **Key study**
Chronic toxicity to *Chironomus* species – Degradate MHPC					
OECD 219 Static 28 d GLP compliant	*Chironomus riparius* (chironomid)	MHPC (methyl-3-hydroxyphenylcarbamite) Purity 98.5 % (w/w)	<u>Emergence ratio</u> **NOEC:** 32 mg/L (nom)[3] **EC_{10}:** 29.1 mg/L (nom)[3] <u>Development rate (male)</u> **NOEC:** 32 mg/L (nom)[3] <u>Development rate (female)</u> **NOEC:** 18 mg/L (nom)[3] **EC_{10}:** 53.78 mg/L (nom)[3]		2013 RAR B.9.2.5.3/05 M-452588-02-1
Chronic toxicity to green algae – Degradate MHPC					
OECD 201 Static 96 h GLP	*Pseudokirchneriella subcapitata* (green algae)	MHPC (methyl-3-hydroxyphenylcarbamite) Purity 93.4 % (w/w)	<u>Growth rate reduction</u> **NOEC:** 6.25 mg/L (nom)[3] **EC_{10}:** 25.2 mg/L (nom)[3]	The actual temperature recordings were not reported and the pH of the test medium increased more than	2000 RAR B.9.2.6.1/06 M-197967-01-

compliant				1.5 units required in OECD TG 201.	1
OECD 201 Static 72 h GLP compliant	*Selenastrum capricornutum* (green algae) (formerly known as *Selenastrum capricornutum*)	MHPC (methyl-3-hydroxyphenylcarbamite) Purity >98 % (w/w)	Biomass growth inhibition **EC_{10}:** 12.3 mg/L (nom)[3] **NOEC:** 10 mg/L (nom)[3] Growth rate reduction **EC_{10}:** 21.5 mg/L (nom)[3] **NOEC:** 22 mg/L (nom)[3]		1998 RAR B.9.2.6.1/07 M-493444-01-1
Chronic toxicity to aquatic macrophytes – Phenmedipham					
ASTM guideline E 1415-91 (1991) Semi-static 14 d GLP compliant	*Lemna minor* (duck weed)	Phenmedipham (technical) Purity 99.4 % (w/w)	Biomass growth inhibition **7 d EC_{10}:** 0.022 mg/L (geo)[4] **7 d NOEC:** 0.024 mg/L (geo)[4] Growth rate reduction **7 d EC_{10}:** 0.044 mg/L (geo)[4] **7 d NOEC:** 0.024 mg/L (geo)[4]	pH of the test medium increased more than 1.5 units required in OECD TG 201. Geometric mean concentrations were only calculated for 7 d endpoints	2004 RAR B.9.2.7/02 M-493457-01-1
OECD 239 Semi-static 14 d GLP compliant	*Myriophyllum spicatum* (Eurasian watermilfoil)	Phenmedipham (technical) Purity 97.7 % (w/w)	Biomass growth inhibition **EC_{10}:** 0.028 mg/L (geo)[4] **NOEC:** 0.0128 mg/L (geo)[4] Growth rate reduction **EC_{10}:** 0.0208 mg/L (geo)[4] **NOEC:** 0.0128 mg/L (geo)[4]	The pH of the test solution was purposely decreased to lower level than recommend in the TG 239 in order to prevent the hydrolysis as much as possible.	2017 RAR B.9.2.7/06 M-580251-02-1 **Key study**
Chronic toxicity to aquatic macrophytes – Degradate MHPC					
OECD 221 Static 7 day GLP compliant	*Lemna gibba* (duck weed)	MHPC (methyl-3-hydroxyphenylcarbamite) Purity 97.5 % (w/w)	Growth rate reduction **NOEC:** 4.5 mg/L (nom)[3]		2013 RAR B.9.2.7/03 M-451664-02-1
Chronic toxicity to aquatic macrophytes – Degradate m-toluidine					
OECD 221 Semi-static 7 day GLP compliant	*Lemna gibba* (duck weed)	m-toluidine Purity 99.1 % (w/w)	Growth rate reduction **NOEC:** 3.05 mg/L (nom)[3]		2015 RAR B.9.2.7/04 M-512630-01-1

[1] mm = mean measured concentration
[2] twa = time-weighted mean measured concentration
[3] nom = nominal concentrations
[4] geo = geometric mean concentrations
* According to the RAR, the study was conducted generally in line with the test method.

11.6.1 Chronic toxicity to fish

Two early life stage toxicity studies for phenmedipham and degradate MHPC were considered valid in the RAR. The studies followed 210 "Fish, Early-life Stage Toxicity Test" (1992) and were conducted on *Oncorhynchus mykiss* (rainbow trout). The experimental NOEC values of 0.041 mg/L and 2.74 mg/L were determined separately for a sum of phenmedipham and degradate MHPC and pure degradate MHPC, respectively. The endpoints are presented in table (**Table 42**) above and the studies are summarized below.

Test results from two prolonged fish test were also presented in the RAR but not evaluated as, according to the CLP guidance, tests consistent with OECD test guideline 210 (or equivalent) should be used for evaluating long-term aquatic hazards. Therefore, those studies are not presented in this CLH proposal.

Study 1 – Phenmedipham

RAR B.9.2.2.1/01 (2014). Early life stage toxicity of phenmedipham to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions.

Early life stage toxicity of phenmediphan to *Oncorhynchus mykiss* (rainbow trout) was studied in 92-day (60 days post hatch) flow-through test conducted according to harmonized version of guidelines OECD TG 210 (1992), US EPA OCSPP 850.1400 (1996) and US EPA-FIFRA OPP 72–4 (1982) and in compliance with GLP. Four replicates, each with 35 eggs at experimental start and thinned to 15 alevins after hatch, were exposed to water control, solvent control and nominal (mean measured concentrations of 0.025 (0.024), 0.050 (0.041), 0.100 (0.096), 0.200 (0.181) and 0.400 (0.361) mg/L. The eggs and fish were observed daily (excluding weekends and holidays).

Dissolved oxygen concentrations ranged from 64 to 102 % oxygen saturation, the pH values ranged from 7.8 to 8.1 and the water temperature ranged from to 10.3 to 11.1 °C in all aquaria over the whole testing period. Developing embryos/larvae were shielded from light exposure until one week post hatch. Fish were exposed in a photoperiod of 16 hours light and 8 hours darkness. The concentration of the test material in the test medium was determined as a sum of phenmedipham and degradate MHPC from two alternating replicate test vessels by HLPC measured in the beginning of the study and at least weekly thereafter. Mean measured recoveries were within the range of 87 to 104 % of the nominal concentrations.

The validity with regards to hatching success (> 75 %) and post hatch success (> 75 %) were fulfilled. Dissolved oxygen was > 64 % of the air saturation value and the water temperature did not differ by more than 1.5° C. Flow-through test design maintained the phenmedipham concentration ≥ 80 % nominal. On study days 0 and 29, the total hardness was slightly out of the range (68 mg $CaCO_3$/L) stated in the protocol (40 – 60 mg $CaCO_3$/L). However, this deviation is not considered to affect the outcome of the study. The 92-day exposure resulted in a NOEC values of 0.041 and 0.096 mg a.s./L for growth and survival, respectively, based on the arithmetic mean measured concentrations of phenmedipham and the metabolite MHPC.

Study 2 – degradate MHPC

RAR B.9.2.2.1/02 (2014). Early life stage toxicity of MHPC to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions.

Early life stage toxicity of degradate MHPC to *Oncorhynchus mykiss* (rainbow trout) was studied in 95-day (62 days post hatch) flow-through conducted to be able to distinguish between the toxicity of parent and degradate. The test was conducted according to harmonized version of guidelines OECD TG 210 (1992), US EPA OCSPP 850.1400 (1996) and US EPA-FIFRA OPP 72–4 (1982) and in compliance with GLP. Four replicates, each with 35 eggs at experimental start and thinned to 15 alevins after hatch, were exposed to control and nominal (mean measured concentrations of 0.625 (0.798), 1.25 (1.26), 2.50 (2.74), 5.00 (5.34) and 10.0 (10.4) mg/L. The eggs and fish were observed daily (excluding weekends and holidays).

Dissolved oxygen concentrations ranged from 91 to 107 % oxygen saturation, the pH values ranged from 7.7 to 7.9 and the water temperature ranged from to 10.5 to 11.0 °C in all aquaria over the whole testing period. Developing embryos/larvae were shielded from light exposure until one week post hatch. Fish were exposed in a photoperiod of 16 hours light and 8 hours darkness. The concentration of the test material in the test

medium was determined from two alternating replicate test vessels by HLPC measured in the beginning of the study and at least weekly thereafter. Mean measured recoveries were within the range of 101 to 128 % of the nominal concentrations.

The validity with regards to hatching success (> 75%) and post hatch success (> 75%) were fulfilled. Dissolved oxygen was > 91 % of the air saturation value and the water temperature did not differ by more than 1.5° C. Flow-through test design maintained the phenmedipham concentration ≥ 80 % nominal. However, on study day 14 recoveries were higher than expected ranging from 118 – 161 % of nominal. The test system was checked for its proper function and additional water samples were taken on study day 15, which were in the expected range. Therefore, this deviation only increased the concentrations and is therefore not considered to affect the outcome of the study. The 95-day exposure resulted in a NOEC value of 2.74 mg a.s./L for (dry weight) growth based on the arithmetic mean measured concentrations of MHPC.

11.6.2 Chronic toxicity to aquatic invertebrates

There is one valid chronic toxicity study available for aquatic invertebrate *Daphnia magna* in the RAR. The lowest chronic toxicity determined in this study was 21 d NOEC 0.005 mg/L for reproduction based on mean measured concentrations of phenmedipham. This is the lowest chronic endpoint and it is used as key study for the long-term aquatic hazard classification of phenmedipham. For degradation products, one 21 day *Daphnia magna* study for m-toluidine and one 28 day *Chironomus riparius* study was considered valid in the RAR.

Study 1 – Phenmedipham

RAR B.9.2.5.1/03 (2014). Chronic toxicity of phenmedipham to *Daphnia magna* under flow-through conditions

Chronic toxicity of phenmedipham (purity 99.1 % w/w) to *Daphnia magna* was studied in 21 d flow-through test following OECD TG 211 and in compliance with GLP. *Daphnia magna* 1st instar <24 hours old neonates were exposed to nominal concentrations of 0.0125, 0.0250, 0.050, 0.10 and 0.20 mg/L, corresponding mean measured concentrations of 0.0114, 0.0235, 0.047, 0.088 and 0.189 mg/L for sum of phenmedipham and degradate MHPC. Four replicates were used per test level containing 5 organisms in each. Sublethal effects, survival (immobilization), time to first brood release, reproduction (living neonates per adult at start of the study) and growth (length and dry weight at study termination) were measured. Dissolved oxygen concentrations ranged from 5.3-8.4 mg/L (58-92 % oxygen saturation), the pH from 8.1-8.3 and the temperature from 19.8-20.4°C during the testing period. Photoperiod was 16 hours light and 8 hours dark with 30 minutes dawn/dusk transition period during the test. The test solutions were analysed to determine the concentration of both phenmedipham, and its degradate MHPC by HPLC-MS/MS. The mean measured concentrations of phenmedipham were 39.2-45.8 % of nominal concentrations. Thus, the endpoints were recalculated by the request of RMS based on arithmetic mean measured concentrations of phenmedipham instead of sum of phenmedipham and MHPC as originally calculated in the study.

Table 43: Summary of relevant information on chronic aquatic toxicity

	Results from the report based on mean measured concentrations of PMP+MHPC µg a.s.+MHPC/L		Recalculated results based on arithmetic mean measured concentratios of PMP µg a.s./L	
	NOEC	LOEC	NOEC	LOEC
Survival (day 21)	47.0	88.0	21.6	45.8
Time to first brood	≥ 189	> 189	≥ 78.4	> 78.4
Living neonates produced per adult	11.4	23.5	5.0	10.5
Adult body length	≥ 189	> 189	≥ 78.4	> 78.4
Adult dry weight	≥ 189	> 189	≥ 78.4	> 78.4

No dose related effects on behaviour or growth were noted for any test level. Survival of adult daphnids ranged from 35-100 % and there were statistically significant differences from the pooled controls in the 0.0235, 0.088 and 0.189 mg/L treatment groups. RMS considered that the immobilizations in the 0.0235 mg/L group was not considered to be treatment related. This statistically significant difference was likely attributable to chance and not a real compound-related effect as there were no sublethal observations of immobilizations in the 0.047 mg/L treatment group. Additionally, the observed immobilization of 20 % corresponds to the criterion of 20 % mortality that can be considered as accidental or inadvertent for the controls as well as for each treatment according to OECD TG 211. This indicates that the performance of the 0.0235 mg/L treatment group would be acceptable. The 21 d NOEC 0.0216 mg/L was determined for survival of adult daphnids based on mean measured concentrations of phenmedipham.

Time to first brood in both the control and solvent control group was day 8 for all replicates. The percent inhibition for neonates per adult as compared to the pooled controls ranged from 9.7-78.3 %, showing statistically significant differences in the four highest treatment groups. **The 21 d NOEC 0.005 mg/L was determined for reproduction based on mean measured concentrations of phenmedipham.**

The study fulfils the validity criteria set in OECD TG 211 and is considered valid in the RAR.

Study 2 – degradate m-toluidine

RAR B.9.2.5.1/04 (2015). Effects of BCS-AU61245 (m-toluidine) on development and reproductive output of the water flea *Daphnia magna* in a static-renewal laboratory test system

Chronic toxicity of degradate m-toluidine (purity 99.1 % w/w) to *Daphnia magna* was studied in 21 d semi-static (3 renewal intervals per week) test following OECD TG 211 and in compliance with GLP. *Daphnia magna* 1st instar <24 hours old neonates were exposed to nominal concentrations of 2.0, 4.0, 8.0, 16.0, 32.0 and 64.0 µg/L, corresponding to time-weighted mean measured concentrations of 1.19, 1.77, 2.87, 4.67, 8.04 and 14.16 µg/L. Ten replicates were used per test level containing one organism in each. The total living offspring per parental animal, the parental age at first offspring emergence as well as the rate of parental survivors and their body-length and dry body mass at the end of the study were recorded. Dissolved oxygen concentrations ranged from 8.6-9.0 mg/L (> 90 % oxygen saturation), the pH from 7.4-7.9 and the temperature from 20.8-21.7°C during the testing period. Photoperiod was 16 hours light and 8 hours dark. For verification of the actual test item concentrations during exposure, water-samples from the start and the end of 3 representative exposure-intervals were analysed by HPLC-UV (LOQ 1.11 µg/L).

The chemical analysis of m-toluidine in the freshly prepared test solutions at start of the chosen exposure intervals revealed recoveries from 101-115% of the corresponding nominal concentrations. Due to degradation of m-toluidine under the current test conditions, the remaining content in aqueous solution at the end of each exposure interval was not detectable, and analytical LOQ of 1.11µg/L was used. Therefore, all

reported results from 21 days of static renewal exposure of *Daphnia magna* to m-toluidine are based on time-weighted mean measured test concentrations.

Table 44: Summary of survival, reproduction and growth of *Daphnia magna* exposed to m-toluidine for 21 days

Treatment µg p.m./L Time-weighted mean measured conc.	Parental endpoints			Reproductive endpoints			
	body length (mm)	dry body mass (mg)	mortality (%)	total offspring per parent animal (n) [1]	parent age at first offspring emergence (days)	total dead offspring (n)	aborted eggs (n)
Control	4.04	0.64	20	97.8	8.66	0	0
1.19	4.16	0.68	20	91.1	9.06	0	0
1.77	4.18	0.71	30	92.7	8.46	0	0
2.87	4.04	0.57	10	89.2	8.76	0	3
4.67	4.14	0.57	10	95.6	9.06	0	0
8.04	3.96	0.61	20	59.8*	9.06	0	14
14.16	3.87	0.54	20	38.1*	9.96*	12	24

[1)] Total number of living offspring produced per parent animal introduced at the start of exposure, reduced by parent animals died accidentally or inadvertently during 21 days of exposure.
* Statistically significant difference from untreated control (verified by Williams Multiple Sequential t-test procedure on a 5% level of significance at one-sided probability).

The study followed the recommendations of OECD TG 211. The validity criteria for control mortality and mean number of living offspring/parent at the end of test were fulfilled and this study is considered valid in the RAR. The 21 d NOEC value of 0.00467 mg/L and 21 d EC_{10} value of 0.00478 mg/L for total offspring produced per parent and the 21 d NOEC value of 0.00804 mg/L for time to first brood were determined based on time-weighted mean measured concentrations of m-toluidine.

Study 3 – degradate MHPC

RAR B.9.2.5.3/05 (2013). *Chironomus riparius* 28-day chronic toxicity test with phenmedipham-MHPC in a water-sediment system using spiked water.

Chronic toxicity of degradate MHPC (purity 98.5 % w/w) to *Chironomus riparius* was studied in 28 d static test according to OECD TG 219 and in compliance with GLP. Groups of 1st instar midges were exposed to the test concentrations of 10, 18, 32, 56 and 100 mg/L and a control (dilution water) with 4 replicate test chambers were maintained in each treatment and control group, with 20 midges in each. Each test chamber contained a quantity of sediment and overlying water. The test chambers were observed three times per week during the first 13 days of the test to make visual assessments of any abnormal behaviour (e.g. leaving sediment, unusual swimming). During the period of expected emergence following day 13, the test chambers were observed on daily basis and the sex and number of fully emerged midges was recorded. The sediment used in the test was initially composed of approximately 0.5% sphagnum peat moss, 20% kaolin clay, and 75% industrial quartz sand. Temperatures in test were within the 20.2±0.09°C range, dissolved oxygen concentrations were ≥8.5 mg/L throughout the test and pH ranged from 8.0-8.4 in the overlying water. Recoveries of MHPC were measured three times during the study, 1 hour, 7 days and 28 days after application in each nominal initial test concentrations and control of the overlying water and the pore water of the sediment.

Analysis of the overlying water at the beginning of the exposure period reflect high recoveries of MHPC with 101% to 109% (mean 105%) of nominal concentrations in all test levels, thus all results and reporting are based on initial nominal concentrations of MHPC in the overlying water. After 7 days of exposure recoveries in the overlying water of 76% to 86% (mean 80%) were found and after 28 days 30% to 74% (mean 56%) of nominal concentrations were detected.

Table 45: Influence on emergence and development rate of *C. riparius* after 28 days exposure to MHPC (based on nominal concentrations of the test item in the overlying water)

Nominal test concentration [mg p.m./L]	Number of introduced midges	Number of emerged midges	Emergence of inserted larvae (pooled sex)			Development rate [1 / d]		
			total [%]	male [%]	female [%]	pooled sex	male	female
control	80	76	95.00	47.50	47.50	0.063	0.067	0.060
10.0	80	73	91.25	45.00	46.25	0.063	0.066	0.060
18.0	80	72	90.00	41.25	48.75	0.062	0.066	0.058
32.0	80	73	91.25	45.00	46.25	0.061	0.066	0.056*
56.0	80	64*	80.00	45.00	35.00	0.058	0.062*	0.054*
100	80	12*	15.00	15.00	0	0.063	0.063*	-

* significant difference ($\alpha = 0.05$)

The 28 d NOEC value of 32 mg/L and EC_{10} value of 29.1 mg/L for emergence ratio for pooled sex, the NOEC 32 mg/L for development rate of males and the 28 days NOEC value of 18 mg/L and EC_{10} value of 53.78 mg/L for development rate of females was determined based on nominal concentrations of MHPC.

The study fulfilled validity criteria set in the OECD TG 219 and is considered valid in the RAR.

11.6.3 Chronic toxicity to algae or other aquatic plants

No valid chronic toxicity data are available for algae, as reliable endpoints were not possible to derive due to rapid hydrolysis of the phenmedipham in available algal studies in the RAR. However, there are valid chronic toxicity studies available for aquatic macrophytes duck weed *Lemna minor* and Eurasian watermilfoil *Myriophyllum spicatum* for phenmedipham (RAR B.9.2.7/02, 2004 & RAR B.9.2.7/06, 2017). For degradate MHPC results of two algal and one duck weed study and for degradate m-toluidine results of one duck weed study are summarised below.

Study 1 – Phenmedipham

RAR B.9.2.7/02 (2004). Phenmedipham - Aquatic plant toxicity test, *Lemna minor*, semi-static, 14 d.

This study is already summarised in chapter 11.5.3 (study 1). The 7 d EC_{10} value of 0.044 mg/L and the 7 d NOEC value of 0.024 mg/L for growth rate of *Lemna minor* were determined based on geometric mean concentrations of phenmedipham. The 7 d EC_{10} value of 0.022 mg/L and the 7 d NOEC value of 0.024 mg/L for biomass growth inhibition were determined based on geometric mean concentrations of phenmedipham. The study fulfilled the validity criteria set in OECD TG 221. The pH-value did increase by more than 1.5 units but RMS considered this not to invalidate the study since the validity criteria for control growth was met and thus RMS considered this study valid in the RAR.

Study 2 – Phenmedipham

RAR B.9.2.7/06 (2017). Toxicity of phenmedipham tech. (BCS-AD17874) to the Aquatic Plant *Myriophyllum spicatum* in a Semi-Static Growth Inhibition Test.

This study *Myriophyllum spicatum* study is already summarised in chapter 11.5.3 (study 2). The lowest 14 d EC_{10} 20.8 µg/L was determined based on growth rate (fresh weight) and on geometric mean measured concentrations of phenmedipham. No EC_{10} could be calculated for growth rate (dry weight) since the control coefficient of variation of this parameter was higher than the respective effect level. The lowest 14 d NOEC value of 12.8 µg/L was determined for the parameters growth rate (fresh and dry weight) and biomass inhibition (dry and fresh weight) based on geometric mean measured concentrations of phenmedipham.

Based on total shoot length biomass inhibition and growth rate, the 14 d NOEC value of 46.4 µg/L and the 14 d EC_{10} value of 28.6 µg/L (biomass inhibition) were determined.

The study was performed according to the OECD TG 239 and the test conditions were in line with the TG, except for the pH which was lower than recommended 7.9. The pH of the test solution was purposely decreased in order to prevent the degradation of phenmedipham as much as possible. Phenmedipham is hydrolytically very unstable in alkaline medium. This is not considered to have effect on the study results since the validity criteria were met. Hence, RMS considered this study valid in the RAR.

Study 3 – degradate MHPC

RAR B.9.2.6.1/06 (2000). Algal inhibition test - *Pseudokirchneriella subcapitata* MHPC (methyl-3-hydroxyphenylcarbamate) Code: AE B038210.

This study is already summarised in chapter 11.5.3 (study 3). The 96 h NOEC value 6.25 mg/L and 96 h EC_{10} 25.2 mg/L for growth rate of algae *Pseudokirchneriella subcapitata* was determined for degradate MHPC based on nominal concentrations. The study fulfilled the validity criteria set in OECD TG 201 for control growth and the coefficient of variation for section-by-section specific growth rate and average specific growth rate during the whole test period in control. However, the study has some limitations, the actual temperature recordings were not reported and the pH of the test medium increased more than 1.5 units required in OECD TG 201. Since the validity criteria for control growth was reached the RMS considered this study valid in the RAR.

Study 4 – degradate MHPC

RAR B.9.2.6.1/07 (1998). Fresh water algal growth inhibition test with methyl-(3-hydroxyphenyl)-carbamate.

This study is already summarised in chapter 11.5.3 (study 4). The 72 h EC_{10} for cell growth inhibition was 12.3 mg/L based on nominal concentrations of degradate MHPC. The NOEC corresponded with 10 mg/L. The 72 h EC_{10} for growth rate reduction of algae *Selenastrum capricornutum* was 21.5 mg/L based on nominal concentrations of degradate MHPC**.** The NOE_rC corresponded with 22 mg/L. The study fulfilled the validity criteria set in OECD TG 201 and is considered valid in the RAR.

Study 5 – degradate MHPC

RAR B.9.2.7/03 (2013). *Lemna gibba* G3 - Growth inhibition test with BCS-AA66045 (phenmedipham-MHPC) under semi-static conditions - Amendment 1 to report.

This study is already summarised in chapter 11.5.3 (study 5). The 7 d NOEC value of 4.50 mg/L was determined for growth rate of frond number and total frond area of plants (*Lemna gibba*) based on nominal concentrations of degradate MHPC. The study fulfilled the validity criteria set in the OECD TG 221 and is considered valid in the RAR.

Study 6 – degradate m-toluidine

RAR B.9.2.7/04 (2015). *Lemna gibba* G3 - Growth inhibition test with BCS-AA61245 (m-toluidine) under semi-static conditions – final report.

This study is already summarised in chapter 11.5.3 (study 6). The 7 d NOEC value of 3.05 mg/L for the growth rate inhibition (frond number and total frond area) of *Lemna gibba* was determined based on nominal concentrations of degradate m-toluidine. The study fulfilled the validity criteria set in the OECD TG 221 and is considered valid in the RAR.

11.6.4 Chronic toxicity to other aquatic organisms

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Aquatic acute toxicity data are available for all the trophic for phenmedipham and its degradates MHPC and m-toluidine. The most acutely sensitive species is aquatic macrophyte, *Myriophyllum spicatum* (Eurasian watermilfoil), with a 14 day $\mathbf{E_rC_{50}}$ and $\mathbf{E_yC_{50}}$ values of **0.0705** and **0.0519 mg/l** based on geometric mean measured concentrations (B.9.2.7/06), respectively.

For acute aquatic hazards, on the basis of this acute aquatic macrophyte endpoint being in the range 0.01 mg/l < $L(E)C_{50}$ ≤ 0.1 mg/l, phenmedipham should be classified as Aquatic Acute 1 (H400) with M-factor of 10.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Degradation

Phenmedipham was not readily biodegradable in two ready biodegradation tests (RAR B.8.2.2.1/01, 1989) & (RAR B.8.2.2.1/03, 1985). Biodegradation of phenmedipham was 34.1 % (ThOD) and 54.3 % (DOC) after 14 days in RAR B.8.2.2.1/01 (1989) and 23-31 % (ThOD) after 35 days in RAR B.8.2.2.1/03 (1985). The pass level of the ready biodegradation test (70 % DOC removal or 60 % theoretical oxygen demand) within 10 days from the onset of biodegradation in the Guidance on the Application of the CLP criteria (ECHA 2017) was not achieved.

Hydrolytic degradation of phenmedipham was strongly pH dependent. Phenmedipham undergoes rapid hydrolysis with the half-lives of few minutes in alkane (pH > 9) and few hours and days in neutral conditions and it was hydrolytically stable in the acidic conditions (RAR B.8.2.1.1/03, 2003), (RAR B.8.2.1.1/04, 2004) & (RAR B.8.2.1.1/05, 2015). In the Guidance on the Application of the CLP criteria (ECHA 2017) data on hydrolysis might be considered for classification purposes only when the longest half-life determined within the pH range of 4.0-9.0 is shorter than 16 days, and it can be demonstrated that the hydrolysis products do not fulfil the criteria for classification as hazardous for the aquatic environment. Phenmedipham was rapidly hydrolysed in the neutral and alkaline conditions but the half-lives were well over 16 days in the acidic conditions. From the hydrolysis products m-toluidine (CAS Number 108-44-1) has harmonised classification Aquatic Acute 1 and it also fulfils the classification criteria for Aquatic Chronic 1 according to the study RAR B.9.2.5.1/04 (2015).

There is one surface water simulation test available (RAR B.8.2.2.2/01, 2013). Phenmedipham was rapidly degraded with the half-lives of 0.01-0.04 days. Mineralisation to CO_2 reached its maximum of 15 % AR after 63 days for [methyl aniline-UL-^{14}C]phenmedipham, thus it cannot be demonstrated that phenmedipham was ultimately degraded, and the half-lives mainly represent primary degradation of phenmedipham. Primary degradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment according to CLP. In this case the degradation product m-toluidine (CAS Number 108-44-1) fulfils the classification criteria for hazardous to the aquatic environment, as indicated above. Degradation of phenmedipham in sterile systems supports the conclusion that phenmedipham was rapidly hydrolysed abiotically under the test condition.

Consequently, phenmedipham is considered to be **not rapidly degradable** because:

- it was not readily biodegradable in ready biodegradation tests.
- hydrolytical degradation half-lives were not under 16 days in the whole pH range of 4.0-9.0 and the hydrolysis product m-toluidine fulfils the classification criteria as hazardous for the aquatic environment.
- it was not demonstrated that phenmedipham is ultimately degraded >70 % within 28 days in the aquatic environment and the degradation product m-toluidine fulfils the classification criteria as

hazardous for the aquatic environment. In the surface water simulation test primary degradation was fast but the mineralisation to CO_2 was at maximum 15 % AR.

Soil and sediment degradation data are not needed for evaluating the rapid degradability criterion in this case as preferred data types are available (i.e., ready biodegradability, hydrolysis, and surface water simulation test). However, as soil and water/sediment simulation tests are available, they are included as supporting data. Aquatic sediment simulation studies showed rapid dissipation but did not fulfil the rapid degradability criterion of CLP as maximum mineralisation to CO_2 was 54 % AR after 98 days (RAR B.8.2.2.3/05, 2015). Half-lives in soil for phenmedipham ranged from 4 to 53.2 days with the maximum mineralisation to CO_2 25.5 % AR after 224 days (RAR B.8.1.1.1.1/02, 1976). The results from aquatic sediment and soil simulation studies support the conclusion that phenmedipham is not rapidly degradable according to the CLP criteria.

Bioaccumulation potential

The whole fish BCF was 121-321 for rainbow trout RAR B.9.2.2.3/01 (1990) and 165 for bluegill sunfish RAR B.9.2.2.3/02 (1988), which are below the trigger value of ≥500 L/kg according to CLP criteria. The BCFs are based on total ^{14}C residual radioactivity. In rainbow trout study RAR B.9.2.2.3/01 (1990) phenmedipham was almost totally hydrolysed and therefore the results mainly represent the bioaccumulation potential of the degradation product MHPC. The whole fish BCF 165 in bluegill sunfish study RAR B.9.2.2.3/02 (1988) was considered more reliable as the rate of hydrolysis and thus the degradation of phenmedipham was less significant. In this classification proposal it is used to determine the bioaccumulation potential of phenmedipham. BCF value obtained in the study was determined using the total ^{14}C residues in viscera, edible and non-edible tissue compared to the total ^{14}C residues in the water. The guidance on the CLP criteria (ECHA 2017) states that the BCF from radio-labelled studies should, preferentially, be based on the parent compound. If these are unavailable, for classification purposes, the BCF based on total radio-labelled residues can be used. Total radioactivity measurements potentially reflect the presence of the parent substance as well as possible degradation product(s) and/or metabolite(s). Thus, BCF values determined by use of radio-labelled test substances are therefore normally overestimated. Nevertheless, BCF value of 165 in the whole fish was under the trigger value of ≥500 L/kg of the CLP criteria. In summary, based on the total ^{14}C residue BCF value and the criteria set out in CLP, phenmedipham has **a low potential to bioaccumulate**.

A measured valid n-octanol/water partition coefficient is available and revealed a log Pow of 2.7 at 20±1°C (RAR B.2.7/01, 2012) and it does not meet the CLP criteria (log Kow ≤4). It is noted that n-octanol/water partition coefficients determined for the major degradation products MHPC and m-toluidine also indicate low potential to bioaccumulate (log Pow <1.6). Experimentally derived BCF values are more preferred data than experimentally derived n-octanol/water partition coefficients in the CLP guidance (2017). Thus, the experimentally determined log Pow values support the conclusion based on BCF <500 L/kg that phenmedipham has a low potential to bioaccumulate.

Long-term aquatic hazard

There are adequate chronic toxicity data available for fish, aquatic invertebrates and aquatic plants for phenmedipham, covering all three trophic levels. The lowest valid chronic toxicity for fish is 92 d NOEC value of 0.041 mg/L for *Oncorhynchus mykiss*. The lowest chronic toxicity for aquatic invertebrates is 21 d NOEC value of 0.005 mg/L for reproduction of *Daphnia magna* and for aquatic macrophytes 14 d NOEC value of 0.0128 mg/L for biomass growth inhibition and growth rate reduction and 14 d EC_{10} value of 0.0208 mg/L for biomass growth inhibition of *Myriophyllum spicatum.*

For degradate MHPC the lowest chronic toxicity is NOEC value of 2.74 mg/L for fish (*Oncorhynchus mykiss*) and therefore it does not fulfil the classification criteria for hazardous to the aquatic environment based on chronic toxicity as there are also data available for all three trophic levels for MHPC.

The lowest chronic toxicity value for m-toluidine is 21 d NOEC value of 0.00478 mg/L for *Daphnia magna*. Thus, m-toluidine fulfils the classification criteria set out in CLP for hazardous to the aquatic environment based on chronic toxicity.

Since phenmedipham is non-rapidly degradable and adequate chronic toxicity data are available for all trophic levels, phenmedipham can be classified according to the criteria set out in CLP in Table 4.1.0(b)(i). In this case classification of Aquatic Chronic 1 is applicable for phenmedipham based on the lowest **NOEC** value of **0.005 mg/l** for *Daphnia magna* (≤ 0.1 mg/l) with a chronic M-factor of 10 (0.001 < NOEC ≤ 0.01 mg/l).

For long-term aquatic hazards, phenmedipham should be classified according to Regulation EC 1272/2008 as Aquatic Chronic 1 (H410) with M-factor of 10.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Conclusions on classification and labelling for environmental hazards of phenmedipham.

Hazard Class and Category code(s)	M factor	Hazard Statement
Aquatic Acute Category 1, H400	10	Very toxic to aquatic life
Aquatic Chronic Category 1, H410	10	Very toxic to aquatic life with long lasting effects

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Phenmedipham is a non-systemic contact herbicide and acts only via the foliage of emerged weeds. Root uptake is nearly excluded as phenmedipham is strongly absorbed by the soil and is fixed in the upper 5 cm.

The substance currently is classified in Annex VI of the CLP Regulation as Aquatic Acute 1; H400 and Aquatic Chronic 1; H410. The DS proposes to retain this classification and add an M-factor of 10 for both acute and chronic hazards.

Below, a summary of the studies included in the CLH report is provided. Only relevant and valid studies for the proposed classification of phenmedipham have been included from the RAR and CLH report.

Degradation

Hydrolysis

In the CLH Dossier, there are 3 studies where hydrolysis of phenmedipham is investigated.

In RAR B.8.2.1.1/03, the abiotic hydrolysis was investigated following OECD TG 111 in a sterile aqueous buffer at pH 4, 5, 7, and 9, following application of [amino Phenol-UL-14C]phenmedipham at a nominal concentration of 3 mg/L. Phenmedipham was hydrolysed at pH 4, 5, 7, and 9 to MHPC. The half-life of phenmedipham in aqueous buffers was calculated assuming first order kinetics. Half-lives were 259 days at pH 4, 47 days at pH 5, 12 hours at pH 7 and 7 min at pH 9.

In the study RAR B.8.2.1.1/04, the abiotic hydrolysis of phenmedipham was investigated following OECD TG 111 in a sterile aqueous buffer at pH 4, 5, 7, and 9, following application of

[amino Phenol-UL-14C] and [methyl aniline-UL-14C]phenmedipham at the test concentration of 0.9 mg/L. The hydrolytic degradation of phenmedipham was strongly depended on the pH of the solution with slower degradation observed at lower pH. The mean calculated half-lives of phenmedipham assuming first order kinetics were 142 days, 18.5 days, 3 hours and 2 minutes at pH 4, 5, 7 and 9, respectively. Phenmedipham hydrolysed to two degradation products, MHPC and m-toluidine.

In the study RAR B.8.2.1.1/05, the hydrolysis of phenmedipham was investigated following OECD TG 111 at 20 °C in sterile aqueous buffer solutions at pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 in the dark for 30 days. The study was performed with [amino-Phenol -UL-14C]phenmedipham at nominal test concentration of 2.73 mg/L. The mean calculated half-lives of phenmedipham ranged from 1 011 days at pH 4.0 to 3.0 hours at pH 8.0. phenmedipham was hydrolytically relatively stable under acidic conditions (pH 4.0 and 4.5), labile in slightly acidic (5.0, 5.5 and 6.0) and neutral conditions (pH 6.5), and undergoes rapid hydrolysis above pH 7.

In addition, there are two studies with the degradant MHPC, which showed hydrolytical stability at different pHs.

Photochemical degradation

Phenmedipham was photochemically stable in the available studies (RAR B.8.2.1.2/01) and (RAR B.8.2.1.2/02) so direct photodegradation in water may contribute to a very limited extend to the degradation of phenmedipham in the environment.

Ready biodegradation

Two studies assess ready biodegradation of phenmedipham: RAR B.8.2.2.1/01 and RAR B.8.2.2.1/03.

Biodegradation of phenmedipham was 34.1 % (ThOD) and 54.3 % (DOC) after 14 days in RAR B.8.2.2.1/01. In this test, phenmedipham was investigated for its biodegradability in test concentration of 25 mg/250 mL in the Modified MITI-Test (I) following OECD TG 301C during 14 days (plateau phase was reached) in three replicates.

In test RAR B.8.2.2.1/03, the ready biodegradability of phenmedipham was studied following a Modified MITI-Test (I) following OECD TG 301C with a nominal concentration of 100 mg/L using an activated sludge. The degradation of phenmedipham after blank correction was between 23 % and 31 % of its ThOD after 35 days. Degradation of phenmedipham started on day 16 in both flasks. A plateau had not been reached by day 35. The results of the test were complicated by an inhibitory effect of phenmedipham on nitrification.

Inherent and enhanced ready biodegradability tests

The inherent biodegradability was studied using phenmedipham according to the OECD TG 302C during 28 days. Inherent biodegradation of phenmedipham was 39.5 % (BOD/ThOD) and 19.2 % (DOC) after 28 days (RAR B.8.2.2.1/02, 1990).

Water degradation data

In the study RAR B.8.2.2.2/01, the Degradation of [methyl aniline-UL-14C]phenmedipham and [amino Phenol-UL-14C]phenmedipham were studied according to OECD TG 309 in surface water under aerobic conditions in the dark for 63 days at 20.9 ± 0.2 °C. The pH in the water ranged from 7.50 to 8.74. Application rates were 0.1 and 0.01 mg/L. The experiment was also

performed in addition under sterile condition at the high concentration (0.01 mg/L).

Primary degradation of phenmedipham was very fast. A single first order kinetics (SFO) DT_{50} value for methyl aniline labelled phenmedipham was 0.04 days and SFO DT_{50} value for amino Phenol labelled phenmedipham was 0.01 days and 0.04 days for low and high concentrations, respectively. Mineralisation was low, not higher than 15 % at day 63 for [methyl aniline-UL-14C] phenmedipham and 13.2 % AR at day 63 for [amino Phenol-UL-14C] phenmedipham. Two degradation products were found. SFO DT_{50} value of 499 days was evaluated for MHPC and SFO DT_{50} value of 47.6 days for m-toluidine.

Water-sediment degradation data

The degradation of [amino Phenol-UL-14C]phenmedipham (purity > 99 %) and [methyl aniline-UL-14C]phenmedipham (purity 97 %) (RAR B.8.2.2.3/02) was investigated in two different water/sediment systems (sandy loam silt and sand) under aerobic conditions over a period of 126 days at 20 °C with an initial phenmedipham concentration of 0.32 mg/L. The water pH in the systems were 6.9 and 7.0. The study was performed in line with OECD TG 308.

Mineralisation to CO_2 was 29.8-34.1 % at day 126 during the degradation of amino-Phenyl labelled phenmedipham. CO_2 formation was 12.6-13.8 % at day 70 during the degradation of methyl aniline labelled phenmedipham. Non-extractable residues in the sediment after 126 days were 50.8-55.3 % AR for amino-Phenyl labelled phenmedipham and after 70 days 69.7-73.4 % AR for methyl aniline labelled phenmedipham. The primary degradant was MHPC which accounted for approximately 1 % at day 126 in both systems. The dissipation half-lives in the water phase for [Amino Phenol-UL-14C]phenmedipham were first order multi-compartment (FOMC) DT_{50} of 0.16 and SFO DT_{50} 0.11 days and in total system FOMC DT_{50} of 0.069 and 0.15 days in sandy loam silt and sand systems, respectively. The half-lives of MHPC were SFO DT_{50} of 9.2-15.3 days in total system and SFO DT_{50} values of 11.6-13.8 days in the water phase.

In the study RAR B.8.2.2.3/03, the degradation of [amino Phenol-UL-14C]phenmedipham was studied in a water/sediment system, applied at a field rate of 4.9 kg a.s./ha, pH 6.0-6.50. This study was conducted according to OECD TG 308.

Mineralisation to CO_2 was low, 13.2 % at the end of the study period (84 days). The main degradant detected was MHPC, which accounted for 0.3 % at the end of the study period. Bound residues represented 74.8 % after 84 days. [Amino Phenol-UL-14C]phenmedipham half-life in the total system was FOMC DT_{50} of 0.023 days and dissipation half-life was FOMC DT_{50} of 0.012 days in water phase.

In the study RAR B.8.2.2.3/04, the degradation of phenmedipham was investigated according to OECD TG 308 in three freshwater water/sediment systems (silt loam, sand and sandy loam) using two labels, [amino Phenol-UL-14C]phenmedipham and [methyl aniline-UL-14C]phenmedipham, at 20 °C in the dark for up to 127 days and a pH ranging from 6.1 to 8.5. The application rate was 0.1 mg/L.

Phenmedipham degraded very quickly with MHPC and m-toluidine being the major degradation products. Mineralisation was not higher than 31.2 % for any of the labelled phenmedipham. Bound residue formation reached a maximum of 52.1 %. The half-lives for [amino Phenol-UL-14C]phenmedipham were calculated following the FOCUS degKinetics (RAR B.8.2.2.3/01). The half-lives in the total system were FOMC DT_{50} of 0.0001 and 0.0007 days in the silt loam and sand systems, respectively, and SFO DT_{50} of 0.35 days in the sandy loam system. The half-lives for MHPC were calculated between SFO DT_{50} of 8.7-21 days in the total system and SFO

DT_{50} of 8.8-20.2 days in water phase.

In the study RAR B.8.2.2.3/05, the aerobic degradation of [amino Phenol-UL-14C]phenmedipham and [methyl aniline-UL-14C]phenmedipham was investigated according to OECD TG 308 in river and a pond water-sediment systems at 20.7 ± 2 °C in the dark for 98 days. pH values were 8.35 and 7.83. The nominal application rate was based on the maximum field application rate of 1 kg a.s./ha.

Amino Phenol-UL-14C]phenmedipham: phenmedipham was completely dissipated after day 1 in the water phase and it was not detected in the sediment of test systems at any sampling point. Mineralisation to CO_2 was 24.2 % maximum. Non-extractable residues in sediment were, in proportion, 65.6 % and 68.6 % AR after 98 days. The only major degradant was MHPC, which reached, in the total system, at the end of the study 5.1 % AR. Two additional degradation products were identified in the pond system, namely 3-[(methoxycarbonyl) amino]Phenyl(3-hydroxyPhenyl)carbamate (M1) and 3-aminoPhenol (M2). Both reached maximum amounts of 0.5 % AR.

The half-lives for the parent in the total system was 0.12-0.09 days for the river and pond system respectively. The degradant MHPC had half-lives SFO DT_{50} of 13.3 and 17.6 days in total system in river and pond, respectively.

Methyl aniline-UL-14Cphenmedipham was not detectable from day 3 onwards in the water phase of the river and the pond test systems. One major degradant (m-toluidine) was found representing 2.0 % at day 40 in water and 2.9 % in sediment day 7. Mineralisation to CO_2 accounted for 54.8 and 13 % AR for phenmedipham in the river and the pond systems at day 98, respectively. Non-extractable residues in sediment were, in proportion, 31.8 % and 67.7 % AR after 98 days.

The degradation half-life for methyl aniline labelled phenmedipham was SFO DT_{50} of 0.21 and 0.14 days in total system for river and pond, respectively. The degradant m-toluidine had half-lives SFO DT_{50} of 1.5 and 4.7 days in total system in river and pond, respectively.

Soil degradation data (including simulation studies)

There were four soil degradation studies included in the CLH report. Half-lives in soil for phenmedipham ranged from 4 to 53.2 days with the maximum mineralisation to CO_2 25.5 % AR after 224 days.

Conclusion on degradation

Phenmedipham is considered to be not rapidly degradable, for classification purposes, because:

- it is not readily biodegradable;
- hydrolysis degradation half-lives were not under 16 days in the whole pH range of 4.0-9.0 and the hydrolysis product m-toluidine fulfils the classification criteria as hazardous for the aquatic environment;
- it was not demonstrated that phenmedipham is ultimately degraded > 70 % within 28 days in the aquatic environment and the degradation product m-toluidine fulfils the classification criteria as hazardous for the aquatic environment. In the surface water simulation test, primary degradation was fast but the mineralisation to CO_2 was at maximum 15 % AR.

Bioaccumulation

In the study RAR B.9.2.2.3/01, juvenile fish (*Oncorhynchus mykiss*) were exposed in a flow-through system to a nominal concentration of [amino Phenol -UL-14C]phenmedipham of 0.02 mg/L (115 fish) and 0.2 mg/L (106 fish) for 64 hours. The test was done according to OECD TG 305.

In the test, phenmedipham was rapidly hydrolysed to MHPC (more than 86 % radioactivity after 21 and 68hours). Therefore the results mainly represent the bioaccumulation potential of the degradation product MHPC. A BCF of 321 for the low concentration and 121 for the high concentration was obtained. The BCF was not lipid normalised or corrected for fish growth and thus BCF could different depending on the test fish lipid content and/or fish growth during the test period.

In the study RAR B.9.2.2.3/02, juvenile fish (*Lepomis macrochirus*) were exposed in a flow-through system to methyl-Phenol ring and Phenyl ring labelled phenmedipham with nominal concentration of 0.03 mg/L for 10 days according to OECD TG 305.

In the test, a BCF value of 165 for the whole fish was obtained. The BCF was not lipid normalised or corrected for fish growth and thus BCF could be different depending on the test fish lipid content and/or fish growth during the test period.

A measured valid n-octanol/water partition coefficient is also available Log K_{ow} of 2.7 at 20 ± 1 °C (RAR B.2.7/01) and it does not meet the CLP criteria (Log K_{ow} ≤ 4). It is noted that n-octanol/water partition coefficients determined for the major degradation products MHPC and m-toluidine also indicate low potential to bioaccumulate (Log K_{ow} < 1.6).

Conclusion on bioaccumulation

The DS concluded that based on BCF and Log K_{ow} values the substance has a low potential to bioaccumulate.

Aquatic toxicity

The next two tables provide a summary of the most relevant acute and chronic studies provided for phenmedipham in the CLH Dossier.

***Table**: Acute aquatic toxicity*

Method	Species	Test material	Results[1]	Remarks	Reference
Acute toxicity to fish - phenmedipham					
OECD TG 203; US EPA OPPTS 835.1075 GLP	*Oncorhynchus mykiss* (rainbow trout)	phenmedipham technical (purity 97.7 % w/w)	**LC_{50}:** 1.84 mg/L (mm)[1]	Validity criteria met	2016 dRAR B.9.2.1/XX M-564852-01-1
Acute toxicity to *Daphnia magna* - phenmedipham					
OECD TG 202; JMAFF 12 Nounan No. 8147 GLP	*Daphnia magna* (cladoceran)	phenmedipham (technical) Purity 97.4 % (w/w)	**EC_{50}:** 2.033 mg/L (mm)[1]	Validity criteria met	2004 B.9.2.4.1/05 M-233654-01-1
Acute toxicity to *Americamysis bahia* – phenmedipham					
OPPTS 850.1035 GLP	*Americamysis bahia* (mysid shrimp)	phenmedipham (technical) Purity 99.1 % (w/w)	**EC_{50}:** 0.23 mg/L (mm)[1]	Validity criteria met	2010 B.9.2.4.2/01 M-409871-01
Acute toxicity to aquatic macrophytes – phenmedipham					

ASTM guideline E 1415-91 (1991) GLP	*Lemna minor* (duck weed)	phenmedipham (technical) Purity 99.4 % (w/w)	**7d EC_{50} (biomass):** 0.109 mg/L (geo)[2] **7d EC_{50} (growth rate):** >0.157 mg/L (geo)[2](geo)[2]	Validity criteria met	2004 dRAR B.9.2.7/02 M-493457-01-1
OECD TG 239 GLP	***Myriophyllum spicatum* (Eurasian watermilfoil)**	phenmedipham (technical) Purity 97.7 % (w/w)	**EC_{50} (biomass):** 0.0519 mg/L (geo)[2] **EC_{50} (growth rate):** 0.0705 mg/L (geo)[2](geo)[2]	Validity criteria met	2017 dRAR B.9.2.7/06 M-580251-02-1 **Key study**

[1] mm = mean measured concentration
[2] geo = geometric mean concentrations

Table: *Chronic aquatic toxicity*

Method	Species	Test material	Results	Remarks	Reference
Chronic toxicity to fish – phenmedipham					
OECD TG 210; US EPA OCSPP 850.1400; US EPA-FIFRA OPP 72–4 GLP	*Oncorhynchus mykiss* (rainbow trout)	phenmedipham technical (purity 99.1 % w/w)	**$NOEC_{(fry\ survival)}$:** 0.096 mg/L (mm)[1] **$NOEC_{(percent\ hatch)}$:** 0.361 mg/L (mm)[1] **$NOEC_{(percent\ swim\text{-}up)}$:** 0.181 mg/L (mm)[1] **$NOEC_{(standard\ length\ growth)}$:** 0.041 mg/L (mm)[1] **$NOEC_{(dry\ weight\ growth)}$:** 0.096 mg/L (mm)[1] **$NOEC_{(morphological\ and\ behavioural\ effect)}$:** 0.041 mg/L (mm)[1]	Validity criteria met	2014 dRAR B.9.2.2.1/01 M-481742-01-1 **Key study**
Chronic toxicity to *Daphnia magna* - phenmedipham					
OECD TG 211; USEPA OCSPP 850.1300 GLP	***Daphnia magna* (cladoceran)**	phenmedipham (technical) Purity 99.1 % (w/w)	**$NOEC_{(reproduction)}$:** 0.005 mg/L (mm)[1] **$NOEC_{(survival)}$:** 0.026 mg/L (mm)[1]	Validity criteria met	2014 dRAR B.9.2.5.1/03 M-482048-01-1 **Key study**
Chronic toxicity to aquatic macrophytes – phenmedipham					
ASTM guideline E 1415-91 (1991) GLP	*Lemna minor* (duck weed)	phenmedipham (technical) Purity 99.4 % (w/w)	Biomass **7d EC_{10}:** 0.022 mg/L (geo)[2] **7d NOEC:** 0.024 mg/L (geo)[2] Growth **7d EC_{10}:** 0.044 mg/L (geo)[2] **7d NOEC:** 0.024 mg/L (geo)[4]	Validity criteria met	2004 dRAR B.9.2.7/02 M-493457-01-1
OECD TG 239 GLP	*Myriophyllum spicatum* (Eurasian watermilfoil)	phenmedipham (technical) Purity 97.7 % (w/w)	Biomass **EC_{10}:** 0.028 mg/L (geo)[2] **NOEC:** 0.0128 mg/L (geo)[2]	Validity criteria met	2017 dRAR B.9.2.7/06 M-580251-02-1 **Key study**

			Growth **EC_{10}:** 0.0208 mg/L (geo)[2] **NOEC:** 0.0128 mg/L (geo)[2]		

[1] mm = mean measured concentration
2 geo = geometric mean concentrations

Acute toxicity to fish

One acute toxicity test with phenmedipham, three with the degradant MHPC and one with degradant m-toluidine on different fish species were considered valid in the RAR.

In the study RAR B.9.2.1/XX, the acute toxicity of phenmedipham to *Oncorhynchus mykiss* (rainbow trout) was studied in 96-hour semi-static test conducted according to OECD TG 203 (1992) and US EPA OPPTS 835.1075 (1996) and in compliance with GLP. Ten fish in each group, were exposed to water control, solvent control and nominal concentrations of 0.128, 0.282, 0.620, 1.36 and 3.00 mg a.s./L, corresponding to the geometric mean measured concentrations of 0.117, 0.250, 0.600, 1.24 and 2.73 mg a.s./L.

The 96h LC_{50} value of 1.84 mg a.s./L was based on geometric mean measured concentration of phenmedipham.

For the degradant MHPC, experimental LC_{50} values of ≥ 75 mg/L were determined using *Oncorhynchus mykiss*, *Cyprinus carpio* (common carp) and *Pimephales promelas* (fathead minnow). For the degradant m-toluidine, an experimental LC_{50} value of 93.3 mg/L was determined in *Cyprinus carpio*. In this test, satisfaction of validity criteria and test substance stability were not reported.

Acute toxicity to invertebrates

One acute toxicity study for water flea *Daphnia magna* (RAR B.9.2.4.1/05 (2004)) and mysid *Americamysis bahia* (RAR B.9.2.4.2/01 (2010)) were considered valid in the RAR for phenmedipham. The lowest toxicity was 96h EC_{50} value of 0.23 mg/L for *Americamysis bahia* based on mean measured concentrations. For the degradant MHPC, two acute toxicity studies for *Daphnia magna* and for degradant m-toluidine one *Daphnia magna* study were considered valid in the RAR. In the last one the EC_{50} = 0.1 mg/L.

In the study RAR B.9.2.4.1/05, the acute toxicity of phenmedipham to *Daphnia magna* was studied in 48h semi-static test according to OECD TG 202 and in compliance with GLP. *Daphnia magna* (< 24 hours old) were exposed to nominal concentrations of 0.00625, 0.0625, 0.625, 1.25, 2.50, 5.00 and 10.0 mg/L at pH 6.0-6.5 in 4 replicates of 5 daphnids in each.

The 48h EC_{50} was determined to be 2.033 mg/L for phenmedipham based on mean measured concentrations.

In the study RAR B.9.2.4.2/01, the acute toxicity of phenmedipham to *Americamysis bahia* was studied in 96h flow-through test according to OPTTS guideline 850.1035 and in compliance with GLP. Juvenile *Americamysis bahia* (20 per treatment level) were exposed to nominal test concentrations 0.063, 0.13, 0.25, 0.50, 1.0 and 2.0 mg/L. The mean measured test concentrations were 0.028, 0.10, 0.23, 0.48, 0.88, 2.0 (non-centrifuged) and 1.9 mg/L (centrifuged).

The 96h EC_{50} was determined to be 0.23 mg/L for phenmedipham based on mean measured concentrations.

There were two studies with Daphnia magna with degradant MHPC RAR B.9.2.4.1/06 and RAR

B.9.2.4.1/07. Both studies were done according to OECD TG 202. The 48h EC_{50} were 14 mg/L based on nominal concentrations and 25.6 mg/L based on mean measured concentrations, respectively.

In the study RAR B.9.2.4.1/08, the acute toxicity of the degradant m-toluidine (purity 99.1 % w/w) to *Daphnia magna* was studied in a 48h static test according to OECD TG 202 and in compliance with GLP. *Daphnia magna* 1st instars were exposed to nominal concentrations of 0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/L, corresponding to mean measured test concentrations of 0.0175, 0.0342, 0.0684, 0.142, 0.295 and 0.582 mg/L.

The 48h EC_{50} was determined to be 0.1 mg/L based on mean measured concentrations.

Chronic toxicity to fish

In the study RAR B.9.2.2.1/01, the toxicity of phenmedipham to *Oncorhynchus mykiss* was studied in 92-day (60 days post hatch) flow-through test conducted according to OECD TG 210 (1992), US EPA OCSPP 850.1400 (1996) and US EPA-FIFRA OPP 72-4 (1982) and in compliance with GLP. Four replicates, each with 35 eggs at experiment start and thinned to 15 alevins after hatch, were exposed to nominal (mean measured concentrations) of 0.025 (0.024), 0.050 (0.041), 0.100 (0.096), 0.200 (0.181) and 0.400 (0.361) mg/L. The concentration of the test material in the test medium was determined as a sum of phenmedipham and degradant MHPC. Mean measured recoveries were within the range of 87 to 104 % of the nominal concentrations.

The 92-day exposure resulted in NOEC values of 0.041 and 0.096 mg a.s./L for growth and survival, respectively, based on the arithmetic mean measured concentrations of phenmedipham and the metabolite MHPC. The same NOEC would apply to phenmedipham.

In the study RAR B.9.2.2.1/02, toxicity of the degradant MHPC to *Oncorhynchus mykiss* was studied in 95-day (62 days post hatch) flow-through. The test was conducted according to OECD TG 210 (1992), US EPA OCSPP 850.1400 (1996) and US EPA-FIFRA OPP 72–4 (1982) in compliance with GLP. Four replicates, each with 35 eggs at experiment start and thinned to 15 alevins after hatch, were exposed to control and nominal (mean measured concentrations) of 0.625 (0.798), 1.25 (1.26), 2.50 (2.74), 5.00 (5.34) and 10.0 (10.4) mg/L. The 95-day exposure resulted in a NOEC value of 2.74 mg a.s./L for (dry weight) growth based on the arithmetic mean measured concentrations of MHPC.

Chronic toxicity to invertebrates

There is one valid chronic toxicity study available for aquatic invertebrate *Daphnia magna* in the RAR (B.9.2.5.1/03) with a 21d NOEC 0.005 mg/L for reproduction based on mean measured concentrations. This is the lowest chronic endpoint and it is used as the key study for the long-term aquatic hazard classification of phenmedipham. For degradation products, one 21d Daphnia magna study for m-toluidine and one 28d *Chironomus riparius* study were considered valid in the RAR.

In the study RAR B.9.2.5.1/03, the chronic toxicity of phenmedipham to *Daphnia magna* was studied in 21d flow-through test following OECD TG 211 and in compliance with GLP. *Daphnia magna* 1st instars were exposed to nominal concentrations of 0.0125, 0.0250, 0.050, 0.10 and 0.20 mg/L. The mean measured concentrations of phenmedipham were 39.2-45.8 % of nominal concentrations and the endpoints were recalculated by the request of the RMS based on arithmetic mean measured concentrations of phenmedipham instead of sum of phenmedipham and MHPC, as originally calculated in the study. A NOEC 0.005 mg/L was

determined for reproduction based on mean measured concentrations.

In the study RAR B.9.2.5.1/04, the chronic toxicity of m-toluidine to *Daphnia magna* was studied in 21d semi-static test following OECD TG 211 and in compliance with GLP. *Daphnia magna* 1st instar neonates were exposed to nominal concentrations of 2.0, 4.0, 8.0, 16.0, 32.0 and 64.0 µg/L, corresponding to time-weighted mean measured concentrations of 1.19, 1.77, 2.87, 4.67, 8.04 and 14.16 µg/L. A NOEC value of 0.00467 mg/L for total offspring per parent animal and an EC_{10} = 0.00478 mg/L for time to first brood were determined based on TWA.

In the study RAR B.9.2.5.3/05, the chronic toxicity of the degradant MHPC to *Chironomus riparius* was studied in 28d static test according to OECD TG 219 and in compliance with GLP. The substance degraded more than 80 % during the test. The 28d NOEC value of 32 mg/L and EC_{10} value of 29.1 mg/L for emergence ratio for pooled sex, the NOEC of 32 mg/L for development rate of males, the 28 days NOEC value of 18 mg/L, and EC_{10} value of 53.78 mg/L for development rate of females were determined based on nominal concentrations of MHPC.

Toxicity to algae or other aquatic plants

During the Peer Review of phenmedipham the validity of available algae studies was discussed and studies where the geomean measured concentrations were calculated based on mean initial concentrations and LOQ/2 were not considered valid. Hence, studies with no intermediate samples with measurable residues were not considered valid and were not included in the CLH dossier. The above two studies with *Lemna minor* and *Myriophyllum spicatum* were considered valid in the CLH dossier (RAR B.9.2.7/02 and RAR B.9.2.7/06).

In the study RAR B.9.2.7/02, the toxicity of phenmedipham to *Lemna minor* was studied in 14d semi-static test according to ASTM guideline E 1415-91 and in compliance with GLP. *Lemna minor* were exposed to initial mean measured concentrations of 1.76, 0.020, 0.048, 0.11, 0.28, 0.69 and 1.76 mg/L.

The 7d EC_{50} value of > 0.157 mg/L for growth rate and the 7d EC_{50} value of 0.109 mg/L for biomass growth inhibition was determined. For chronic toxicity, the 7d EC_{10} = 0.044 mg/L and the 7d NOEC = 0.024 mg/L for growth rate of *Lemna minor*. The 7d EC_{10} value of 0.022 mg/L and the 7d NOEC value of 0.024 mg/L for biomass growth inhibition were determined. Results are based on geometric mean concentrations. The study fulfilled the validity criteria set in OECD TG 221.

In the GLP test RAR B.9.2.7/06, the toxicity of phenmedipham to *Myriophyllum spicatum* was studied in 14 d semi-static test according to OECD TG 239 except for the pH, which was lower than recommended 7.9. The pH of the test solution was purposely decreased in order to prevent the hydrolysis of phenmedipham as much as possible. Shoots of *Myriophyllum spicatum* were exposed via the water phase to nominal concentrations of 3.0, 9.49, 30.0, 94.9 and 300 µg/L of test item. The corresponding geometric mean measured concentrations were 0.898, 3.85, 12.8, 46.4 and 170 µg/L. Endpoints were provided based on geomean concentrations.

For growth rate, the most sensitive parameter was the 14d EC_{50} value of 0.0705 mg/L. For chronic endpoints, biomass growth inhibition resulted in an EC_{10} of 0.028 mg/L and a NOEC of 0.0128 mg/L. Growth rate reduction resulted in an EC_{10} of 0.0208 mg/L and a NOEC of 0.0128 mg/L.

For the degradant MHPC, two valid algal and one duckweed study were available in the RAR. For degradant m-toluidine one valid duck weed study was available in the RAR (RAR B.9.2.7/04 (2015)). All of the metabolites were observed to be less toxic than the parent.

Conclusion of the Dossier Submitter (DS)

Aquatic acute toxicity data are available for all the trophic levels for phenmedipham and its degradants MHPC and m-toluidine. The most acutely sensitive species is aquatic macrophyte *Myriophyllum spicatum* with a 14 day E_rC_{50} of 0.0705 mg/L based on geometric mean measured concentrations.

For acute aquatic hazard, on the basis of this acute aquatic macrophyte endpoint being in the range 0.01 mg/L < $L(E)C_{50}$ ≤ 0.1 mg/L, the DS proposed that phenmedipham should be classified as Aquatic Acute 1; H400 with an M-factor of 10.

Phenmedipham is considered to be not rapidly degradable and to have a low bioaccumulative potential. There are adequate chronic toxicity data available for all three trophic levels. The lowest valid chronic toxicity for fish is 92d NOEC value of 0.041 mg/L for *Oncorhynchus mykiss*. The lowest chronic toxicity for aquatic invertebrates is 21d NOEC value of 0.005 mg/L for reproduction of *Daphnia magna* and for aquatic macrophytes a 14d NOEC value of 0.0128 mg/L for biomass growth inhibition and growth rate reduction and a 14d EC_{10} value of 0.0208 mg/L for biomass growth inhibition of *Myriophyllum spicatum*. Thus, a classification of Aquatic Chronic 1; H410 is applicable for phenmedipham, according to the DS, based on the lowest NOEC value of 0.005 mg/L for *Daphnia magna* (≤ 0.1 mg/L) with a chronic M-factor of 10 (0.001 < NOEC ≤ 0.01 mg/L).

Comments received during public consultation

Three MSCAs commented during public consultation, one of which agreed with the proposed classification.

Another MSCA asked if an E_rC_{10} (dry weight) endpoint was available for the *Myriophyllum spicatum* study. It also asked if a statistically based EC_{10} might be more appropriate given the steep toxicity profile. Furthermore, they asked if measurements of test item concentrations in sediment were available to support the use of water phase concentrations which declined over the study period pinpointing that this is important to consider exposure routes.

The DS answered that a growth rate E_rC_{10} (dry weight) value of 0.0048 mg/L was calculated in the original *Myriophyllum spicatum* study report. However, the control coefficient of variation of this parameter was higher than the respective effect level. Thus, the EC_{10} endpoint for growth inhibition (dry weight) is not considered reliable.

The DS further indicated that measurements were only available in water. According to the DS, the observed loss of test item during the study occurred mainly because of hydrolytic degradation of phenmedipham and the shoots of *Myriophyllum spicatum* were exposed via water phase and, thus, they considered that water phase is relevant exposure route.

RAC took note of the fact that a reliable EC_{10} dry weight cannot be obtained although it cannot check the raw data. RAC considered that the *Myriophyllum* test is adequate for classification.

The third MSCA agreed with the proposed classification. Yet since the metabolite m-toluidine 21d NOEC value of 0.00478 mg/L for *Daphnia magna* appears to be more toxic than the parent, they asked if this value should be considered for chronic classification.

The DS reviewed the test available both for parent and metabolite and answered that toxicity of the parent substance phenmedipham and degradant m-toluidine is within the same order of magnitude for aquatic invertebrates, and both toxicity values would result in the same classification of Aquatic Chronic 1 with a chronic M-factor of 10. Nevertheless in this case they

preferred to classify phenmedipham according to the lowest toxicity value for the parent substance (21d NOEC 0.005 mg/L).

RAC agreed in using phenmedipham data for classification since for the parent there is full a data set whereas for the metabolite there is no chronic data for fish.

Assessment and comparison with the classification criteria

RAC agrees with the DS that the data on phenmedipham and not its metabolites should be used for classification since the most reliable and complete data set is available for the parent compound, which is not less toxic than any of the degradation products.

Acute toxicity

Aquatic acute toxicity data are available for all three trophic levels for phenmedipham. The lowest acute endpoints are:

- Fish: *O. mykiss* 96h LC_{50} = 1.84 mg a.s./L
- Invertebrates: *Americamysis bahia* 96h EC_{50} = 0.23 mg/L
- The most acutely sensitive species is the aquatic macrophyte, *Myriophyllum spicatum* with a 14 day E_rC_{50} of 0.0705 mg/L based on geometric mean measured concentrations. RAC considers that the water sediment *Myriophyllum* test is suitable for classification for various reasons (although exposure via sediment cannot be totally ruled out): the substance is a herbicide acting only via the foliage of emerged weeds and *Myriophyllum* has been demonstrated to be the most sensitive acute species, application of the test substance is done via the water column and substance concentration reduces mainly because of hydrolysis.

Hence, according to the Classification criteria, phenmedipham warrants classification as **Aquatic Acute 1; H400, M-factor 10** (0.01 mg/L < $L(E)C_{50}$ ≤ 0.1 mg/L).

Chronic toxicity

RAC agrees with the DS that phenmedipham in **not rapidly degradable**:

- The substance it is not readily biodegradable;
- Hydrolytical degradation half-lives were not under 16 days in the whole pH range of 4.0-9.0 and the hydrolysis product m-toluidine fulfils the classification criteria as hazardous to the aquatic environment;
- It was not demonstrated that phenmedipham is ultimately degraded > 70 % within 28 days in the aquatic environment (under neutral and alkaline conditions, phenmedipham undergoes fast primary degradation with a half-life below 16 days) and the degradation product m-toluidine fulfils the classification criteria as hazardous for the aquatic environment.

With a BCF of 165 below the trigger value of 500 and a Log K_{ow} of 2.7, below the trigger value of 4, RAC agrees with the DS and considers that phenmedipham has **a low potential to bioaccumulate.**

There are adequate chronic toxicity data available for all three trophic levels:

- Fish: *Oncorhynchus mykiss* 91d NOEC = 0.041 mg/L
- Invertebrates: *Daphnia magna* 21d NOEC = 0.005 mg/L (there is no chronic data with the acute most sensitive species for invertebrates *A. bahia;* a chronic study with *A. bahia* might potentially lead to a lower NOEC than *Daphnia*)
- Algae or other Aquatic Plants: *Myriophyllum spicatum* 14 days EC_{10} (growth) = 0.0208 mg/L

The lowest chronic toxicity value is the 21d NOEC = 0.005 mg/L for reproduction of *Daphnia magna*. Based on this value and the substance being non-rapidly degradable, a classification of **Aquatic Chronic 1; H410, M-factor of 10** (≤ 0.1 mg/L) with a chronic (0.001 < NOEC ≤ 0.01 mg/L) is warranted.

RAC agrees with the DS that phenmedipham warrants **classification as Aquatic Acute 1; H400, M = 10 and Aquatic Chronic 1; H410, M = 10.**

Supplemental information - In depth analyses by RAC

Toxicity of phenmedipham to invertebrates: phenmedipham is more toxic to invertebrates than the closely related substance Desmedipham, which is most toxic to algae and macrophytes (as can be expected from the mode of action of both substances), without an explanation for this difference in the dossier. However, the metabolite m-toluidine and phenmedipham exhibit toxicity to invertebrates. Although, in the chronic parent test with daphnia m-toluidine was not measured and, instead, MHPC, whose toxicity to invertebrates is low, was measured, a reason for the higher toxicity of phenmedipham to invertebrates could be the metabolites. This assumption can also be supported by the fact that acute toxicity of the parent to Daphnia is lower than that of m-toluidine.

Algae studies with phenmedipham: The CLH dossier indicates that studies where the geomean measured concentrations are calculated based on mean initial concentrations and LOQ/2 are not considered valid and hence not included in the Dossier. However, in accordance with the CLP Guidance, RAC does not consider the lack of intermediate measurements a reason to dismiss studies if they are relevant and reliable.

RAC has reviewed the available algae studies for phenmedipham in the RAR and none of them will be included in this opinion. The study RAR B9.2.6.1/04 fulfils validity criteria and is GLP compliant. In the study, various endpoints are presented; however, it is unknown to RAC which geometric mean concentrations were used to derive such endpoints. Since full replicate raw data is not available, RAC could not determine fully reliable endpoints.

Chronic *Chironomus* test with MHPC: In the study RAR B.9.2.5.3/05, chronic endpoints of degradant MHPC to *Chironomus riparius* were obtained with nominal concentrations. The substance degraded more than 80 % during the test and measured concentrations should have been used. The test will be used as supporting information.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

The hazard class is not assessed in this dossier.

13 ADDITIONAL LABELLING

14 REFERENCES

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15 ANNEXES

Finland, 2017. Revised Renewal Assessment Report (RAR) on phenmedipham prepared by the rapporteur MemberState Finland in the framework of Commission Implementing Regulation (EU) No 844/2012, December 2017