Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

fenpropidin (ISO); (*R*,*S*)-1-[3-(4-tert-butylphenyl)-2-methylpropyl]piperidine

EC Number: -
CAS Number: 67306-00-7

CLH-O-0000007218-72-01/F

Adopted

1 December 2022

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CLH-O-0000007218-72-01/F

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

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

Chemical name: **fenpropidin (ISO); (*R*,*S*)-1-[3-(4-tert-butylphenyl)-2-methylpropyl]piperidine**

EC Number: **-**

CAS Number: **67306-00-7**

The proposal was submitted by the **Czech Republic,** co-submitted by **Germany,** and received by RAC on **25 August 2021.**

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

PROCESS FOR ADOPTION OF THE OPINION

The **Czech Republic** has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **6 September 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **5 November 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Anja Menard Srpčič**

Co-Rapporteur, appointed by RAC: **Wendy Rodriguez, supported by Annemarie Losert**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **1 December 2022** by **consensus**.

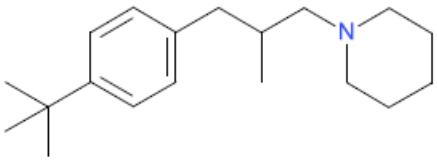
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	fenpropidin (ISO); (*R*,*S*)-1-[3-(4-tert-butylphenyl)-2-methylpropyl]piperidine	-	67306-00-7	Repr. 2 Acute Tox. 4 Acute Tox. 4 STOT SE 3 STOT RE 2 Eye Dam. 1 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H361d H332 H302 H335 H373 (nervous system) H318 H317 H400 H410	GHS08 GHS07 GHS05 GHS09 Dgr	H361d H332 H302 H335 H373 (nervous system) H318 H317 H410		M = 1000 M = 100	
RAC opinion	TBD	fenpropidin (ISO); (*R*,*S*)-1-[3-(4-tert-butylphenyl)-2-methylpropyl]piperidine	-	67306-00-7	Repr. 2 Acute Tox. 4 Acute Tox. 4 STOT SE 3 STOT SE 3 STOT RE 2 Skin Irrit. 2 Eye Dam. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H361d H332 H302 H335 H336 H373 (nervous system, eyes, lungs) H315 H318 H317 H400 H410	GHS08 GHS07 GHS05 GHS09 Dgr	H361d H332 H302 H335 H336 H373 (nervous system, eyes, lungs) H315 H318 H317 H410		oral: ATE = 1330 mg/kg bw M = 1000 M = 10000	
Resulting Annex VI entry if agreed by COM	TBD	fenpropidin (ISO); (*R*,*S*)-1-[3-(4-tert-butylphenyl)-2-methylpropyl]piperidine	-	67306-00-7	Repr. 2 Acute Tox. 4 Acute Tox. 4 STOT SE 3 STOT SE 3 STOT RE 2 Skin Irrit. 2 Eye Dam. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H361d H332 H302 H335 H336 H373 (nervous system, eyes, lungs) H315 H318 H317 H400 H410	GHS08 GHS07 GHS05 GHS09 Dgr	H361d H332 H302 H335 H336 H373 (nervous system, eyes, lungs) H315 H318 H317 H410		oral: ATE = 1330 mg/kg bw M = 1000 M = 10000	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Fenpropidin is a piperidine derivative (structurally related to the morpholine fungicides), composed of the racemic mixture of two enantiomers, and used as an agricultural fungicide[1] to control powdery mildews, rusts and *Rynchosprium secalis* in cereal. At 25°C, fenpropidin is a yellow liquid with a log 4ers of 0.83, 2.9 and 4.5 at pH 4.2, 7 and 9 respectively.



Fenpropidin was included in Annex I of Council Directive 91/414/EEC (Commission Directive 2008/66/EC of 30 June 2008) in 2009 and is an approved active substance under Regulation (EC) 1107/2009 (PPPR). The CLH proposal for fenpropidin was submitted by the Czech Republic and Germany in a combined Draft Renewal Assessment Report (DRAR) and CLH report. This report relied entirely on the dossier prepared by Syngenta Crop Protection AG and ADAMA Agriculture B.V. (Fenpropidin Task Force led by Syngenta) submitted for the active substance, fenpropidin, and a formulated product Tern 750 EC.

The formulated product Tern 750 EC is a water-emulsifiable concentrate containing 750 g/L of fenpropidin. The specification of purity of the active substance is 960.0 g/kg. According to the Safety Dara Sheet (SDS) of Syngenta, the pH of Tern 750 EC varies between 8 and 12 (1 % w/v). In an earlier DAR (2005, volume 3) submitted by Sweden, fenpropidin (purity more than 99%) is described as a relatively strong base, with a pKa estimated to be 10.1.

The CLH report discusses relevant new studies or information submitted and evaluated since the inclusion of fenpropidin in Annex I to Council Directive 91/414/EEC. Some of the studies contained in the DAR (2005) were not mentioned in the CLH report. Several of them were performed with an old formulation A-7516B, which is identical to the current variant A-7516D (Code number of the actual marketed form, TERN™), except that the mixture of aromatic hydrocarbons used for A-7516D was naphthalene depleted (see DAR 2005, volume 4). The two formulations are considered toxicologically relevant for the acute toxicological potential and therefore, were considered for the classification proposal. No additives were mentioned for the current formulation whereas significant (>1 g/kg) impurities were stated (see the CLH report). Genotoxicity studies performed with these impurities were negative (see DRAR 2020, volume 4).

Toxicokinetics

In a study (Anonymous 1994a), fenpropidin absorption, excretion, tissue distribution and blood kinetics were studied in rats after oral exposure (0.5 or 100 mg/kg bw). In addition, biliary

[1] The mode of action identified was an inhibition of ergosterol biosynthesis by imitating the carbocationic high-energy intermediates (HEIs) of the conversions catalysed by the enzymes sterol C14-reductase and sterol C8-isomerase of the post-squalene part of ergosterol biosynthesis. (Krauß *et al.*, 2021).

excretion after oral exposure in female rats (0.5 mg/ kg bw) and excretion after IV injection in both males and females (0.5 mg/ kg bw) were investigated. Fenpropidin is rapidly and extensively absorbed after a single oral exposure of 0.5 mg/kg bw in rats (> 80%). At higher oral exposure (100 mg/kg bw), there is a difference between male and female absorption (93% in males and 58% in females). The Cmax was reached from 30 min (at the low dose in both sexes) to 1h (high dose males) or 2h (high dose females) after administration. The absorbed dose is distributed mainly to the liver and kidney, the principal organs of metabolism and excretion, and the half life in these tissues is between 3 and 22 hours (males). No tissue accumulation was observed. Rapid excretion is described, with 77 to 97% of the dose excreted during the first 24h in both the oral and intravenous administration groups. In all groups, the male rats excreted 86-93% of the total administered radioactivity in the urine, and the rest eliminated via the faeces. At the low dose, female rats eliminated 80 and 16% of the dose via urine and faeces, respectively. At the high dose the urinary excretion decreased to 57% of the dose; correspondingly the amount excreted in the faeces increased to 39%. Only a negligible amount of radioactive CO2 was detected (after oral and IV exposure).

The metabolism of fenpropidin is extensive and no parent compound is observed in the excreta (urine, bile or faeces) after oral and IV administration (0.5 and 100 mg/kg bw) (Muller *et al*., 1994). In the study by Molitor *et al.* (1996), two metabolic pathways were proposed for fenpropidin in rats and found to be qualitatively independent of the dose level (oral 0.5 and 100 mg/kg), the route of administration (intravenous and oral, 0.5 mg/kg) and pre-treatment (0.5 mg/kg for two weeks). Nevertheless, there were some quantitative sex-dependent differences (see below). The main pathway proceeded by oxidation of one of the tertiary butyl groups to alcohol (CGA289268). Further oxidation of the alcohol leads to the major metabolite in urine, the propionic acid form (CGA289267). Further oxidation of a second methyl group gives a hydroxy propionic acid derivate (SYN515213). In bile and faeces, the major metabolite is a sulphate conjugate of CGA289268. In a minor pathway, the piperidine ring is oxidated and cleaved to yield the methyl-propyl-amino pentanoic acid derivative (SYN522216). Subsequent oxidation reactions result first in the formation of the respective methyl-propyl-amino propionic acid (SYN522215), the methyl-propyl-amine (SYN515215) and ultimately the benzoic acid derivative (CGA294973). The methyl-propyl-amino pentanoic acid (SYN522216) and the methyl-propyl-amine (SYN515215) undergo oxidation at the tert-butyl moiety to produce the respective propionic acid derivatives SYN522217 and SYN515216. These can be further oxidised at the tertiary butyl groups to the corresponding propyl alcohols and the corresponding propionic acid derivatives (see Figure 2.6.1.1-1 in the CLH report). The principal urine metabolite is CGA289267, accounting for 46-79% of the administered dose (both sexes). In female rats, the predominant faeces metabolite amounted to 6-27% of the dose while in males, this metabolite accounted for less than 1%. Other metabolite fractions in urine, faeces and bile do not exceed 2.5% of the administered dose (metabolic pattern: 14 fractions in urine and 16 fractions in faeces, Muller *et al.*, 1994).

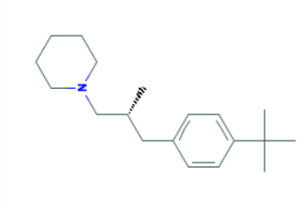
It should be noted in an *in vitro* comparative study performed on rats, dogs and human cryo-preserved hepatocytes (Sayer *et al.*, 2017, GLP compliant) that the metabolism seems to be much more extensive in rats than in humans, with an intermediate profile in dogs. The proportion of chromatographic radioactivity attributable to the parent compound (Table 2.6.1.1-1 of the CLH report) decreased from 98.7% and 97.6% (0 h) to 0.76% and 2.44% (4h) in male and female rat samples, respectively; and from 96.1% and 99.4% to 59.2% and 32.6% (4h) in male and female dog samples, respectively. On the other hand, in human samples, the proportion of chromatographic radioactivity attributable to the parent compound decreased from 97.0% (0h) to 89.7% (4h) only. Nevertheless, some uncertainties were noted in this study. Unlike rats and dogs, human hepatocytes were from mixed gender, therefore a potential gender-based difference in metabolism cannot be detected. In addition, the variation between individuals is unknown. M10, described as the most notable metabolite in human, was found in the same proportion in

humans (7.8%) as in dogs (6.2-9.2%), but was lower in rats (1.5-0.6%). The identity of M10 is not provided.

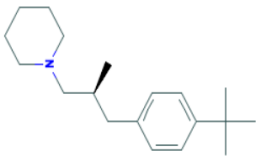
Two dermal absorption studies were available in the DAR, 2005 (Kunz *et al.*, 2003; Roper and Sherratt, 2003). The Kunz *et al.* (2003) study shows that fenpropidin absorption was moderate in rats after dermal exposure, with a rapid excretion mainly via urine. After 6 hours, 10.0% and 4.3% of the applied low (0.03 mg/cm2) and high (7.5 mg/cm2) dose were absorbed, respectively. The penetration rates were calculated to be 0.44 and 54.4 mg×cm-2×h-1 for the low and high dose level, respectively. Comparing the actual amounts absorbed and as the difference in penetration rates were a factor of 124 lower than the actual concentration difference between the two dose levels, this might indicate that the absorption process was saturated at the high dose. An *in vitro* TERN 750 EC rat and human skin membrane percutaneous penetration study (Roper and Sherratt, 2003) revealed a lower penetration through human than rat epidermis, with a ratio rat to human flux constants of 4.6:1 and 7.7:1 at a low (0.029 mg/cm2) and high concentration (6.84 mg/cm2), respectively.

The structure of both enantiomers of fenpropidin is given below. These are provided below (source Pubchem):

(S)-fenpropidin



I-fenpropidin



RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Explosives

The DS proposed no classification based on the absence of functional groups potentially associated with explosive properties, supported by two negative EU A.14 studies.

Flammable liquid

The flash point of Fenpropidin is 156°C, which is above the classification threshold of 60°C and consequently the DS proposed no classification.

Self-reactive substance or mixture

The DS proposed no classification based on the absence of functional groups potentially associated with explosive and self-reactive properties.

Pyrophoric liquid

The DS proposed no classification based on experience in handling.

Self-heating substances

The DS proposed not classification based on the self-ignition temperature of 265°C as measured using an EU A.15 method.

Substance or mixture which in contact with water emits flammable gas

The DS proposed no classification because fenpropidin fulfils all the screening criteria listed in the CLP Regulation 2.12.4.1.

Oxidising liquids

The DS proposed no classification based on a negative (but not clearly identified) test.

Substance or mixture corrosive to metals

The DS proposed no classification because the substance does not contain strong acid or basic functional groups and is not able to form complexes with metals.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC agrees with the DS assessment for all physical hazard classes except oxidising liquids and self-heating substances.

Oxidising liquids

Fenpropidin does not contain oxygen atoms, thus no classification is warranted.

Self-heating substances

Fenpropidin is a liquid substance therefore its melting point is below 160°C, which is the threshold included in the CLP guidance. Therefore, no classification is warranted.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The CLH report states that a literature search has been performed and that no published cases of poisoning, adverse health effects, investigation nor reporting of health effects on the general population has been associated with fenpropidin exposure.

Acute Oral Toxicity

Two rat acute oral studies are available (Anonymous 1981a and Anonymous 1981b). Both of these studies predated but were compliant with OECD TG 401 - the Anonymous 1981b study was not GLP compliant and, the purity of the substance was not stated. None of these studies provided a dose-mortality curve and slope. The LD50 was calculated as 2173 and 1452 mg/kg for males and females respectively in Anonymous (1981a), while the LD50 values calculated in Anonymous (1981b) were identical for both males and females (2009 mg/kg bw).

The DS proposed to classify fenpropidin for acute toxicity in Category 4; H302. The CLH report did not explicitly propose an ATE but mentioned that based on the acute oral toxicity studies, the LD50 for fenpropidin is in the range between 1452 – 2009 mg/kg bw.

Acute Dermal Toxicity

One guideline compliant (OECD TG 402, GLP) acute dermal study was performed on rats (Anonymous 1993a). No mortalities were observed following a 24 hour application of fenpropidin at 4000 mg/kg bw. No classification was proposed by the DS.

Acute Inhalation Toxicity

One acute inhalation study was performed on rats (Anonymous 1981c). This study predated but was considered compliant with OECD TG 403 (but was non-GLP). The purity of fenpropidin was not reported. The LD50 was calculated as 1.22 mg/L for both males and females. The DS proposed to classify fenpropidin for acute toxicity in Category 4; H332. No ATE was proposed.

Some studies described in the EFSA DAR (2005) were not reported in the CLH report (Arcelin G *et al.*, 2000a; Grunert B *et al.*, 1995 b). These studies have been performed with the A-7516B variant (see "RAC general comments") and have been considered of interest for the current opinion. Therefore they are described in the "assessment and comparison with the classification criteria" section of this opinion.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Acute Oral Toxicity

The acute oral studies available are described in the table below:

Table: *Summary of the Acute oral toxicity studies*

Study, guideline, animal strain, most relevant deviations (if any)	**Test substance**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
Acute oral (gavage), compliant with OECD TG 401 – GLP Rat Outbred albino 10/sex/group Deviations: Dose response	Fenpropidin technical (purity 94%) Vehicle: gum 8ersis 4% aqueous	0, 2.05, 2.34, 2.63, 3.51, 4.68, 5.85 mL/kg in males (corresponding to 1872, 2136, 2401, 3205, 4273, and 5341 mg/kg bw), and 0, 0.59, 1.17, 1.46, 1.61, 1.76,	Calculated LD50 : Males = 2.38 mL/kg (equivalent to 2173 mg/kg bw) Females = 1.59 mL/kg	Anonymous 1981a

curve and confidence limit not reported		2.05, 2.34, 3.51 mL/kg in females (corresponding to 539, 1068, 1333, 1470, 1607, 1872, 2136 and 3205 mg/kg bw) Single dose followed by 14 days observation period	(equivalent to 1452 mg/kg bw) Values between 95% confidence limit	
Acute oral (gavage), compliant with OECD TG 401 – Non GLP Rat Sprague Dawley 10/sex/group Deviations: Dose response curve not given	Fenpropidin technical (purity not stated) Vehicle: distilled water	0, 1.0, 1.6, 2.5, or 4.0 mL/kg bw (corresponding to 913, 1461, 2283 or 3652 mg/kg bw) Single dose followed by 14 days observation period	Males and females = 2.2 mL/kw bw (equivalent to 2009 mg/kg bw) 95% confidence limits: 2.0 to 2.5 mL/kg bw	Anonymous 1981b
Acute oral (gavage), OECD TG 423, GLP Rats HanIbm: WIST (SPF) 3/sex/group Deviations: Loss of weight during acclimatization period (29 and 22 % for males and females respectively)	A-7516 B 82 % w/w of Fenpropidin Vehicle: distilled water	200 mg/kg (males and females) and 2000 mg/kg (females) Single dose followed by 15 days observation period	200 mg/kg bw < LD50 < 2000 mg/kg bw No statistical methods were used	Arcelin *et al.* 2000a

In Anonymous (1981a), animals were exposed to 2.05, 2.34, 2.63, 3.51, 4.68 or 5.85 mL/kg bw of fenpropidin by gavage. The doses were converted in mg/kg bw (see table above). Mortality has been reported from day 1 to 10 at the first doses of 1872 mg/kg bw for 3/10 males and 1333 mg/kg bw for 5/10 females (see table B.6.2.1.-1 from RAR volume 3, 2021). Body weight increments were reduced in males from 1872 mg/kg bw and in females from 1068 and 1607 mg/kg bw (indication of statistical significance not provided). Surviving females appeared to have recovered by study termination while an improvement was visible in males. Tissues from five males and five females from the highest dose groups, and all macroscopic abnormalities, were examined histopathologically (see STOT SE section). The acute oral LD50 value of fenpropidin was calculated (Probit analysis as described by Finney, 1971) to be equivalent to 2173 mg/kg bw for males and 1452 mg/kg bw for females.

In another acute oral toxicity study in rats (Anonymous 1981b), animals were exposed via gavage to fenpropidin at 0, 1.0, 1.6, 2.5, or 4.0 mL/kg bw. The doses were converted to mg/kg bw (see table above). Mortality was observed from day 1 to 7 at the first dose of 2283 mg/kg bw in both males (8/10) and females (8/10). All surviving rats had apparently recovered within five days of dosing. A reduction in bodyweight gain was noted in the first week only, in both males and females (level of statistical significance not provided). The acute oral LD50 value of fenpropidin was calculated (method of Weil, 1952) to be equivalent to 2009 mg/kg bw for both male and female rats. The clinical effects at the low dose and the macroscopic findings are described in the STOT SE section.

In Arcelin *et al.* (2000a), rats were exposed by gavage to 200 mg/kg bw (males and females) or 2000 mg/kg bw (females only) of a mixture containing 82 % w/w of fenpropidin. No mortalities occurred at 200 mg/kg bw but all animals died at 2000 mg/kg bw (two 24 hours after dosing and one due to technical error during administration). No body weight loss attributable to the treatment was recorded (no statistics provided). The clinical effects at the low dose and macroscopic findings are described in the STOT SE section. All surviving rats had recovered from the slight clinical effects within two days of dosing.

Most studies show limitations. The study by Arcelin et al. (2000a) was performed according to OECD TG 423 and GLP, however, in this study a mixture containing only 82% w/w fenpropidin was tested. The full study report refers to raw data for the detailed composition, but these raw data were not accessible to RAC. The study by Anonymous (1981a) pre-dates the OECD TG 401, but was assessed to be compliant to GLP, whereas Anonymous (1981b) is a non GLP study. Anonymous (1981b) should be interpreted with care, as the purity of fenpropidin is not reported. In addition, fenpropidin tends to be hydrophobic. Anonymous (1981a) used gum 10ersis as the vehicle, which appears a more suitable vehicle than distilled water (as used by Arcelin *et al.* (2000a) and Anonymous 1981b), as gum Arabic can emulsify lipophilic compounds, such as fenpropidin.

Although all studies were performed in rats, it cannot be excluded that rats from specific strain or sex can be more sensitive. The LD50 values reported are lower for strains used in Anonymous (1981a) and Arcelin *et al.* (2000a) studies than the one from the Anonymous (1981b) study. Females seem to be more sensitive than males in the Anonymous (1981a) study. This increase in sensitivity was not observed in the Anonymous (1981b) study and only females were tested at highest dose in Arcelin *et al.* (2000a) study. Nevertheless, the Arcelin *et al.* (2000a) study can be considered as supportive of an LD50 below 2000 mg/kg bw for female rats.

Taking all this together, RAC supports the DS proposal to classify fenpropidin for acute oral toxicity in Category 4; H302, based on the recommendation of the CLP guidance that "*classification is based on the lowest ATE values available, i.e the lowest ATE in the most sensitive appropriate species tested*." Nevertheless, although female mortality reaches 100% at all doses $\geqslant$1872 mg/kg bw, the increase is not dose dependent at the lower doses, with 50%, 60% and 40% of mortality at 1333, 1470 and 1607 mg/kg bw, respectively (Anonymous 1981a), see table B.6.2.1.-1 in the DRAR 2021) which. The default ATE of 500 mg/kg bw is considered by RAC to be overprotective, as no mortalities were observed at this dose in any of the studies. Regarding the full data set, an ATE of 1330 mg/kg bw, corresponding to the rounded value of the first dose where 50% of mortality in the most sensitive sex of the most sensitive strain was seen (Anonymous 1981a) is proposed.

RAC supports the DS proposal to **classify fenpropidin for acute oral toxicity in Category 4; H302 with an ATE of 1330 mg/kg bw**.

Acute Dermal Toxicity

The acute dermal studies available are described in the table below:

***Table**: Summary of the Acute dermal toxicity studies*

Study, guideline, animal strain, most relevant deviations (if any)	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
Acute dermal (semi-occlusive), OECD 402, GLP Rat Tif:RAI f (SPF) 5/sex/ group	Fenpropidin technical (purity 97%)	4000 mg/kg bw 24 hour application followed by 19 males or 21 days (females) observation period	> 4000 mg/kg Bw for males/females	Anonymous 1993a
Acute dermal toxicity (occlusive), OECD 402, GLP Sprague-Dawley rats 5/sex/ group	A-7516 B, purity was not stated, undiluted formula	2000 mg/kg bw 24 hour application followed by 14 days observation period	> 2000 mg/kg Bw for males/females	Grunert *et al.* 1995b

In Anonymous (1993a), rats were exposed to fenpropidin at 4000 mg/kg bw during 24h but no mortality occurred. Changes in bodyweight were not consistent and information on statistical significance was not provided. No mortality was detected in rats in the Grunert *et al.* (1995b) study upon exposure to 2000 mg/kg bw. The purity of the test material was not stated. The body weight development appeared to be slightly affected, but no information on statistical significance was provided. The clinical effects at the low dose and macroscopic findings are described in the STOT SE section. In both studies, the rats had recovered from systemic clinical effects within five days of dosing.

RAC agrees with the analysis of the DS. The LD50 of fenpropidin in rats was greater than 2000 mg/kg. **No classification is warranted for acute dermal toxicity.**

Acute Inhalation Toxicity

The acute inhalation study available in the CLH report is described in the table below:

***Table :** Summary of the Acute inhalation toxicity study*

Study, guideline, animal strain, most relevant deviations (if any)	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
Acute inhalation (nose-only), compliant with OECD TG 403, Non-GLP Rat CD (Sprague-Dawley) 8/sex/group Deviation: acclimatisation was only 3 days for some animals	Fenpropidin (purity not reported). Concentration – MMAD: 0.47 mg/L – 1.89 µm, 0.68 mg/L – 1.78 µm 1.09 mg/L – 2.12 µm 1.34 mg/L – 2.32 µm 1.78 mg/L – 2.17 µm 2.39 mg/L – 2.28 µm Mean of particles less than 4 µm between 72 and 83% for all dose groups (less than 8µm=100% in all dose groups)	0, 0.47, 0.68, 1.09, 1.34, 1.78 or 2.39 mg/L (gravimetric concentration). 4 hour exposure, followed by 14 day observation period	1.22 mg/L (95% CL 1.03-1.44 mg/L) Males and females	Anonymous 1981c

In Anonymous (1981c) study, rats were exposed to aerosolised fenpropidin for 4 hours, at gravimetric concentrations of 0.47, 0.68, 1.09, 1.34, 1.78 or 2.39 mg/L. At these concentrations, mortality was 0/7, 1/7, 3/7, 8/8, 7/8, 7/8 and 0/7, 1/7, 2/7, 8/8, 6/8, 8/8 for males and females, respectively. Considering that the gravimetric concentrations were reported to be incorrect at a dose of 1.34 mg/L (this group in fact had the highest concentration of 3.120 mg/L, verified by gravimetric analysis, see DRAR volume 3), the mortality can be considered to be dose-dependent. The LC50 was calculated (Probit analysis method of Finney, 1964) to be 1.220 mg/L with upper and lower 95 % confidence limits of 1.44 and 1.03 mg/L. All treated animals showed reduced weight gain throughout the study, which was dose related (statistical significance not mentioned). The clinical effects at the low dose and macroscopic findings are described in the STOT SE section.

The purity was not reported in this study, which is considered a deficiency, as an impurity or mixture effect cannot be excluded. Nevertheless, the mortality is dose dependent (after concentration correction), with the first deaths occurring at relatively low doses in both males and females and generally occurring from day 1, increasing the concern. In addition, this result is in line with the observed oral acute toxicity. Although the mode of action is unknown, considering the clinical signs observed, it seems reasonable to RAC to assume a systemic action. Therefore, with an LD 50 calculated to be 1.220 mg/L and in absence of any other data that invalidates this result, RAC supports the DS proposal to **classify fenpropidin as Acute Toxicity Category 4 (H332: Harmful if inhaled).**

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

Four studies were considered by the DS as relevant to evaluate for classification of fenpropidin for specific target organ toxicity after single exposure: Anonymous (1981c); Anonymous (1981a); Anonymous (1981b) and Anonymous (1993a) (see the acute toxicity section). The DS is of the opinion that there is no evidence of a specific target organ toxicity in acute oral and dermal toxicity studies. However, though significant lung toxicity was noted only in conjunction with acute mortality in the acute inhalation toxicity study, clinical signs of respiratory tract irritation were already detected at 0.47 mg/L, in the absence of mortality (Anonymous 1981c). Therefore, the DS proposed to classify fenpropidin for STOT-SE in Category 3 (H335: May cause respiratory irritation).

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The studies relevant for STOT SE classification evaluation are described in the table below:

Table: *Summary of the STOT SE classification evaluation relevant studies*

Study, guideline, animal strain, most relevant deviations (if any)	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Acute inhalation (nose-only), compliant with OECD 403, Non GLP Rat CD (Sprague-Dawley) 8/sex/group Deviation: minimum acclimatisation period was 3 days only	Fenpropidin (purity not reported) Aerosol Vehicle: air Nose-only inhalation 0, 0.47, 0.68, 1.09, 1.34, 1.78 or 2.39 mg/L (gravimetric concentration). 4 hour exposure, followed by 14 day observation period	**LD50: 1.22 mg/L** (upper and lower 95 % confidence limits of 1.44 and 1.03 mg/L) For effects at doses greater than the LD50, see Table 32 of the CLH report. Clinical findings: lethargy, prostration, cold to touch, ataxia and respiratory difficulties (all exposed animals); alopecia and skin sores (≥ 0.68 mg/L) Macroscopic examination: patchy darkening of the lungs (from 0.68 mg/L) 1.09 mg/L: Mortality: 3/7 males (days 4-14), 2/7 females (day 1) 0.68 mg/L: Mortality: 1/7 males (day 1), 1/7 females (day 1) 0.47 mg/L: Mortality: 0/7 males, 0/7 females	Anonymous 1981c
Acute oral (gavage), compliant with OECD TG 401, Non GLP Rat Outbred albino 10/sex/group (not all dose levels were applied to both sexes)	Fenpropidin technical (purity 94%) Vehicle: gum Arabic 4% aqueous 0, 1872, 2136, 2401, 3205, 4273, or 5341 mg/kg bw (males) 0, 539, 1068, 1333, 1470, 1607, 1872, 2136, 3205 mg/kg bw (females) Single dose followed by 14 day observation period	LD 50 calculated by the DS: Males = 2173 mg/kg bw Females = 1452 mg/kg bw For effects at doses greater than the LD50, see Table 32 of the CLH report. 1872 mg/kg bw: One male had pneumonitis. 1068 mg/kg bw: One female showed reduced numbers of lymphocytes in the spleen All doses groups: clinical findings: rhinorrhea, dacryorrhea, lethargy, flaccidity, ataxia and piloerection	Anonymous 1981a
Acute oral (gavage), compliant with OECD TG 401, non GLP Rat Sprague Dawley 10/sex/group Deviations: only seven animals from the high dose group	Fenpropidin technical (purity not stated) Vehicle: distilled water 0, 1.0, 1.6, 2.5, or 4.0 mL/kg (corresponding to 913, 1461, 2283 or 3652 mg/kg bw)	LD50 : 2009 mg/kg bw For effects at doses superior of the LD50, see Table 32 of the CLH report. All doses groups: clinical findings: piloerection, hunched posture and lethargy (at doses below LD 50: all males and females)	Anonymous 1981b

were subject to histopathology	Single dose followed by 14 day observation period		
Acute oral (gavage), OECD 423, GLP Rats HanIbm: WIST (SPF) 3/sex/group Deviations: Weight loss during acclimatisation	A-7516 B 82 % w/w active ingredient (Fenpropidin) Vehicle: distilled water 200 mg/kg (males and females) and 2000 mg/kg (females)	200 mg/kg bw < LD50 < 2000 mg/kg bw. All doses groups: clinical findings: slight to marked tremors and uncoordinated movements	Arcelin G et al. 2000a
Acute dermal (semi-occlusive), OECD 402, GLP Rat Tif:RAI f (SPF) 5/sex/ group	Fenpropidin technical (purity 97%) 4000 mg/kg bw 24 hour application followed by 19 (f) or 21 (m) days observation	No mortality occurred No abnormalities at necropsy Clinical findings: piloerection and hunched posture	Anonymous 1993a
Acute dermal toxicity-(occlusive), OECD 402, GLP Sprague-Dawley rats 5/sex/ group	A-7516 B, purity was not stated, undiluted formula 2000 mg/kg bw 24 hour application followed by 14 days observation	No mortalities occurred. No abnormalities at necropsy Clinical findings: Erythema (50% of the rats), slight apathy (one female, during one day). Lower body weight (two females < than 3%).	Grunert *et al.* 1995b

In the acute inhalation toxicity study by Anonymous (1981c), all treated animals showed reduced weight gain throughout the study. Rats that died during, or shortly after exposure had respiratory difficulties and body staining. All animals, including controls had some clinical signs (ruffled wet fur, nasal secretion and facial staining due to lacrimation). In addition to these signs, for groups which experienced less than 50% of mortality (i.e. at doses below 1.22 mg/L), a defined spectrum of clinical signs was observed (severity in direct proportion with the dose received), as lethargy, prostration, cold to touch, ataxia and respiratory difficulties. At 1.094 mg/L, animals were also suffering from dry scaly skin, body sores and skin irritation, leading to vocalisation and aggression. Macroscopic examination revealed dark patches in the lungs (from 0.68 mg/L), alopecia and skin sores (≥ 0.68 mg/L dose group). Microscopic examination was restricted to control and to high dose exposure animals, therefore at lethal level. Increased lung weights (≥1.34 mg/L), pulmonary congestion, oedema, tracheitis, evidence of pulmonary and dermal irritation (≥1.78 mg/L) were also observed.

In the acute oral (gavage) study by Anonymous (1981a), dying animals showed reduced body weight, convulsions, diarrhoea and anorexia. Clinical signs of toxicity such as rhinorrhoea, dacryorrhea, lethargy, flaccidity, ataxia and piloerection were detected in animals exposed, including in groups exposed to doses below LD50 (2173 mg/kg bw in males and 1452 mg/kg bw in females). Information on statistical significance was not provided. In the individual animal data in the full study report, no ataxia nor lethargy were described in control males. The majority of the cases of ataxia and lethargy were reported as moderate in exposed males, but cases of mortality were reported at all doses tested in males. In females, only a few cases of slight lethargy were reported (4/10) in control group, whereas all the females from exposed groups

(below LD50) showed lethargy, mostly with moderate severity. Ataxia was seen in exposed females only. No deaths were reported in females from 539 and 1068 mg/kg bw dose groups. A dose dependent increase in occurrence or severity of lethargy and ataxia was seen at these doses (see "Narcotic effects summary" table below). In some cases, these effects persisted for 3 or 4 days, but had eventually resolved and the animals survived. Pallor of extremities, hypothermia and alopecia were infrequently observed. Surviving animals appeared to have recovered by study termination. All animals were macroscopically examined. Animals that died during the observation period were found with extended stomach, necrotizing gastritis and enteritis, enlarged lymph node and spleen adherent to the stomach. Histopathological examination was performed only on animals that presented macroscopic abnormalities and in 5 animals per sex from the high dose group, in which the concentration was greater than the LD50. Therefore, most of the histological findings were considered to occur at doses too high to be relevant for classification. Nevertheless, at doses below the LD50, one female was found with enlarged spleen, spleen-stomach adhesion and reduced numbers of lymphocytes in the spleen (at 1068 mg/kg bw). Four animals (two males and two females) found dead from highest doses group also showed reduced number of lymphocyte in addition to enlarged lymph nodes or spleen adherence to the stomach. According to the study authors, these abnormalities of lymphatic tissue might be attributed to treatment (no more explanation available). Pneumonitis was detected in one male (1872 mg/kg bw) but is of unknown relevance to RAC as no clear and consistent statement was made in the full study regarding this finding.

In the acute oral (gavage) study by Anonymous (1981b) clinical signs were observed in all males and females exposed, including in groups exposed to doses below the LD50 (2009 mg/kg bw). These observations consisted of piloerection, hunched posture, lethargy and slightly reduced body weight gain. Information on the statistical significance was not provided. Dose dependence was not described and the reporting was poor, but in the individual animal data from the full study report, no lethargy was described in control animals (but only piloerection), whereas all animals at doses below the LD50 manifested lethargy. When mentioned, the severity was evaluated as moderate in all animals. From 1461 mg/kg bw fenpropidin, there were also incidences of diarrhoea, reduced respiratory rate, body tremors, ataxia, reduced locomotor activity and collapsed condition. Surviving rats had apparently recovered within five days after treatment. All animals were examined macroscopically, and terminal autopsy findings consisted of slight adherence between the stomach and spleen in three males and three females treated at doses beyond the LD50 (2283 mg/kg bw). Histopathological examination was performed only in 7 animals per sex in high dose groups (beyond the LD50). Therefore, the histological examination has been considered to have been conducted at too high doses to be relevant for classification.

The main clinical sign reported was tremors and uncoordinated movements in Arcelin *et al.* (2000a) gavage study. This effect was transient, slight to marked and visible at a dose that did not induce mortality (200 mg/kg) in one male. One female presented hunched posture and weak activity in the 200 mg/kg dose group. Tremors and uncoordinated movement were noted in all females at the high dose (except the female who died during administration), but the animals were found dead on test day 2. No treatment related body weight changes nor macroscopic findings were observed. No histopathological examination was mentioned.

No mortality occurred in rats dermally exposed to fenpropidin up to 4000 mg/kg bw in the Anonymous (1993a) study. Clinical signs of piloerection and hunched posture were seen in all animals but did not persist after day 5. Signs of skin irritation were observed but had recovered by day 21 (exact incidences not provided). The animals were subjected to a gross necropsy at the end of the observation period. No deviation from normal morphology was found. No histopathological examination was mentioned.

No mortality occurred in dermal study by Grunert *et al.* (1995b). The only clinical signs reported were erythema (50% of the rats, from day 8), apathy (one female rat, 2000 mg/kg bw), and a very slight reduction in body weight (<3% in two females). The animals were subjected to a gross necropsy at the end of the observation period. No macroscopic organ changes were observed. No histopathological examination was mentioned.

Most of the effects seen in acute oral studies were observed at dose levels above the LD50 for each studies, and above the ATE proposed for oral acute toxicity (1333 mg/ kg bw) therefore it's difficult to exclude that they may have been the cause of death. Nevertheless, some transient effects were noted at lower doses:

- In Anonymous (1981a), rhinorrhea, dacryorrhea, lethargy, flaccidity, ataxia and piloerection were detected in most animals exposed from 539 mg/kg bw, but in none in the control group. One female was found with enlarged spleen, spleen stomach adhesion and reduced numbers of lymphocytes in the spleen (1068 mg/kg bw), a profile which has been seen in four animals that were found dead in highest dose group. Nevertheless, surviving animals had recovered by study termination.
- In Anonymous (1981b), lethargy was seen in exposed males and females (from 913 mg/kg bw exposure group). At doses below the LD50 for this study, there were also incidences of diarrhoea, reduced respiratory rate, body tremors, ataxia, reduced locomotor activity and collapsed condition. These effects were reversible and no occurrences were mentioned in control group.
- One male was reported to present transient tremors and uncoordinated movements after one oral exposure of 200 mg/kg bw of fenpropidin in Arcelin *et al.* (2000a). Although at a lethal concentration, similar effect was reported in the high dose group. No occurrences were mentioned in control group.

Most of the effect described are related to narcosis (see table below). Some effects on lymphatic tissue at a dose below the LD50 are described in one animal in one study (Anonymous 1981a). Histopathology was not performed in all animals, and therefore other cases could have been missed. Nevertheless, with the current data, these finding seem of uncertain relevance for classification.

Narcotic effects summary (observed in absence of deaths)

Study	Exposure route	Effect	Concentration (compared to ATE or LD50 by study/exposure route)	Occurrence in control, dose dependance (if available)
Anonymous 1981a	Oral	Lethargy, ataxia	539 and 1068 mg/kg bw in females (2.5x lower than the ATE for oral exposure and LD50)	No lethargy in male control, 4/10 slight cases in control females (4h). No cases of ataxia in control from both sexes. **In females: 539 mg/kg bw**: 10/10 lethargy (9/10 moderate, 3/10 persist slight lethargy until 2d day), 4/10 ataxia (slight, first day) **1068 mg/kg bw**: 10/10 lethargy (9/10 moderate, 10/10 persists until 3rd day, 7/10 moderate), 10/10 ataxia (4/10 moderate, 4/10 persist until 3rth or 4th day) **=> At non-lethal dose: Increase in incidence and severity with dose in females**

Anonymous 1981b	Oral	Lethargy	913 to 1461 mg/kg bw (1.4x lower than the ATE and 2.2x lower than the LD50)	No cases in control. **Both sexes: 913 mg/kg bw:** 10/10 until day 2, lethargy moderate when mentioned. **1461 mg/kg bw:** 10/10 day 1 only, moderate when mentioned. **=> No dose dependence, reporting poor**
Arcelin *et al.*, 2000a	Oral	Uncoordinated movement	200 mg/kg bw (6.6x lower than the ATE, no LD50 for that study)	No control mentioned. 1/3 Marked tremors and slight uncoordinated movement day 1 only. **=> Only one non-lethal dose**
Anonymous 1993a	Dermal	None	Up to 4000 mg/kg bw (N/A: no lethal dose)	No control mentioned. Dose response N/A as no effect seen.
Grunert *et al.*, 1995b	Dermal	Slight apathy	2000 mg/kg bw (N/A no lethal dose)	No control mentioned. 1/5 female showed apathy. No control reported. **=> Only one tested dose**
Anonymous 1981c	Inhalation	Lethargy, ataxia	0.47 mg/L (2.6 lower than LD50)	No cases of lethargy and ataxia in control. Clear statement of study author that these signs increased in severity in direct proportion with the dose received.

Transient lethargy, ataxia and lack of coordination are criteria for classification as STOT SE3 (narcotic effects) according to the guidance (see Annex I: 3.8.2.2.2). These effects are mentioned in all the oral studies, and no occurrences were noted in the control group except slight lethargy in 4/10 females (no cases in control group males) from Anonymous (1981a) study. These findings in female controls should nevertheless be balanced with the fact that in exposed females, lethargy was described in all animals, with up to moderate severity and persisted for several days before recovering. In addition, no ataxia was seen in males nor females in the control groups whereas an increase of ataxia was also seen at non-lethal doses in exposed females in the same study. Both lethargy and ataxia appeared to be dose-dependent (in occurrence and/or severity) at doses below the LD50. In other oral studies, only one dose was non-lethal, or the reporting was poor, therefore dose dependence cannot be excluded. In addition, apathy was seen in one rat exposed dermally to 2000 mg/kg bw of fenpropidin (Grunert *et al.*, 1995b) and lethargy and ataxia were described in animals after low concentration inhalation exposure ($\geqslant$0.47 mg/L) to fenpropidin (Anonymous 1981c). Although the effects seen in the acute dermal study could be a chance finding (only one rat, and no effect in the other dermal study, although performed with higher dose of fenpropidin, with a good level of purity), the inhalation study reported that for groups below the LD50, the severity of clinical signs increased with the dose. Such effects were not mentioned in respective control groups. Considering the fact that human data are not available, that no toxicokinetic data that would allow to conclude that this effect on rats are not relevant to humans and that there are no guidance values for Category 3 (and therefore that evidence for narcotic effects at any dose level could be considered), RAC considers that the conditions for classification within Category 3 are met.

The Anonymous (1981c) inhalation study also found respiratory difficulties and coldness to touch, already observable at the lowest dose tested, without mortality (0.47 mg/L; 2.6 lower than LD50). At > 0.679 mg/L (1.8 lower than LD50), patchy darkening of the lungs was detected in the animals, suggesting pulmonary irritancy. At doses above the LD50, increased absolute lung weights as well as pulmonary congestion and oedema associated with tracheitis were observed.

Although these effects could be indicative of pulmonary irritancy, they are considered to have occurred at too high doses to be considered relevant for STOT SE3 classification. While Anonymous (1981h) is a 28-day study, it was noted that animals from the mid-dose group (0.08 mg/L; 15.2 lower than LD50) manifested respiratory distress after only a single 6h-exposure to fenpropidin. No deaths attributable to the treatment was reported at this dose. Respiratory distress was also reported in the high dose group and was described as severe. After three exposures, wheezing was observed in both groups. This data is considered as a key finding and relevant for STOT SE 3 classification. In addition, the same study point toward local irritation in the nasal passages in high dose group males and females after one week of exposure (see table in STOT RE section; 28-day inhalation study in rats with fenpropidin: incidence (grading) of nasal passages findings). Skin irritation/eye damage properties of fenpropidin as well as the high pH of fenpropidin were also considered relevant in a weight of evidence approach.

RAC concurs with the DS and considers that the available data on fenpropidin fulfils the criteria for respiratory tract irritation properties of STOT SE3 classification. In conclusion, RAC supports the DS proposal to **classify fenpropidin as STOT SE 3; H336, H335 (narcotic effects, respiratory irritation).**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Two GLP compliant studies in the rabbit are available on the CLH report.

One was performed in male New Zealand White Rabbits (Anonymous 1984) and predated but was considered compliant with OECD TG 404. The deviations were that the purity of fenpropidin was not reported and occlusive dressing instead of semi-occlusive patching was applied. In addition, examinations of the application site were at 45 and 68 hours instead of 48 and 72 hours. Signs of skin irritation and severe oedema were seen in all animals, and skin irritation did not resolve by day 14.

The second study was performed in female New Zealand White Rabbits (Anonymous 1999) and is considered as GLP and OECD TG 404 compliant. Very slight to well-defined erythema and very slight to slight oedema were observed in all animals and these had regressed by day 10.

Fenpropidin is currently notified as a skin irritant in the C&L inventory. The DS proposed not to classify fenpropidin for skin corrosion/irritation.

Some studies described in EFSA DAR (2005) were not reported by the CLH report. Grunert *et al.*, 1995a study has been performed with A-7516 B variant (see "RAC general comments") and has been considered of interest for the current opinion.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The skin corrosion/irritation studies available in the CLH report are described in the table below:

Table : *Summary of the skin corrosion/irritation studies*

Study, guideline, animal strain	**Test substance,**	**Dose levels, duration of exposure**	**Results**	**Reference**
Acute skin Irritation (Occlusive patching), OECD 404 compliant, GLP Rabbit New Zealand white 6 Males	Fenpropidin (purity not reported) – Undiluted substance Control not mentioned	0.5 mL applied to shorn flank, 4 hour application Irritation response at 1 hour, 1, 2 & 3 days after removal of dressings and at intervals for up to 14 days	No mortalities and no signs of toxicity Persistent signs of skin irritation, severe oedema seen in all rabbits. Mean scores for individual tested animals (calculated from scores at 24, 45 and 68 hours): Erythema: 3.0, 3.7, 3.0, 3.0, 4.0, 4.0 Oedema: 4.0, 4.0, 4.0, 4.0, 4.0, 4.0	Anonymous 1984
Acute skin Irritation (semi-occlusive dressing), OECD 404, GLP Rabbit New Zealand white 3 Females	Fenpropidin (purity 99.5%) – Undiluted substance Control patch – distilled water	0.5 mL applied to shorn flank 4 hour application Irritation response at 1 hour, 1, 2 & 3 days after removal of dressings and at intervals for up to 21 days.	No mortalities but slight weight loss in all rabbits Reversible signs of skin irritation, partly reversible scaling Mean scores for individual tested animals (calculated from scores at 24, 48 and 72 hours): Erythema: 2.0, 2.0, 2.0 Oedema: 1.0, 1.3, 1.3	Anonymous 1999
Acute dermal irritation/corrosion (occlusive), OECD 404, GLP Albino rabbits (species not mentioned) 1 male, 2 females	A 7516 B (purity not stated) – Undiluted substance Control not mentioned	0.5 mL/animal 4 hour application	No effects on mortality, clinical signs, behavior, skin reactions. Slight and inconsistent body weight changes	Grunert *et al.*,1995a

In the first study performed on male rabbits (Anonymous 1984), persistent signs of skin irritation were seen in all animals from 1 hour following decontamination. Severe oedema was present in all animals 24 hours after decontamination and persisted until day 6 when moderate skin thickening obscured any oedema. At day 6, all animals had also severe erythema. Additional signs of skin irritation were seen in all animals and included scabbing, hardening, cracking, desquamation, staining and areas of blanched skin. After 48 hours exposure, two animals showed skin reactions indicative of necrosis. However, in both cases the reactions did not persist and therefore necrosis was unlikely. Signs of skin irritation did not resolve by day 14 when the study was terminated (in all but one rabbit), however, no evidence of tissue scarring in any of the animals was observed. Therefore, based on this study fenpropidin seemed to be at least a strong

irritant. Nevertheless, this study has several limitations including that the purity of fenpropidin is not reported and that an occlusive patching was used, which resulted in more rigorous test conditions compared to the semi-occlusive patching required according to the test guideline. According to the CLP guidance, the method of application should be accounted for in the evaluation of effects (especially in borderline cases).

In the second study, performed this time on female rabbits only (Anonymous 1999), the irritation was less severe (see table above). Very slight to well-defined erythema and very slight to slight oedema were observed in all animals, persisting until day 10. Scaling at the application site was detected in all animals on days 10, 14, and 17. All skin reactions had resolved by day 21. Therefore, severity criteria mentioned in the CLP guidance (2017, page 275) is not fulfilled as none of the animals included in the study reached a mean score of 2.3 for erythema/eschar nor oedema. Nevertheless, the CLP guidance mentions that "w*hen inflammation persists to the end of the observation period in 2 or more test animals, taking into consideration alopecia (limited area), hyperkeratosis, hyperplasia and scaling, then a material shall be considered to be an irritant.*" As scaling persisting in all three tested animals until day 17 was observed, the reversibility criteria that warrant classification for skin irritation is fulfilled.

No effects were observed in Grunert *et al.* study (1995a) performed with A 7516 B, except an inconsistent change in body weight. Some limitations are noted: only three animals were tested, purity was not mentioned and exposure was under occlusive conditions.

In this context, it is highlighted that slight to severe erythema, slight oedema and, later on, necrosis (males and females) were detected in rats in the well-performed acute dermal (semi-occlusive) toxicity study by Anonymous (1993a) which followed OECD TG 402. Scaling in females was also observed. Skin lesions had recovered by day 21 only. In the acute dermal toxicity study by Grunert *et al.* (1995b) with occlusive exposure to A 7516 B, erythema was reported on 50% of the rats (OECD TG 402). The animals had not recovered by day 14 (observation period). In the 21 day dermal study in rabbits (Anonymous 1981d; OECD TG 410), severe skin irritation was also observed after occlusive dressing in all animals exposed to 0.2 mg/kg bw/day fenpropidin and greater (severity increased with dose).

Adopting a WoE approach and considering that:

- Non-persistent slight erythema and oedema with presence of persistent scaling until day 17 are detected in all three animals (Anonymous 1999), corresponding to the applicable criterion for skin irritation category 2 from the CLP Regulation;
- Well performed acute dermal studies (OECD TG 402; OECD TG 410) shows several signs of skin irritation, with different levels of severity as erythema, oedema, scaling, lesions and necrosis;
- Fenpropidin is considered as a relatively strong base (pKa=10.1), characteristics that could induce irritation properties.

In contrast to the DS, RAC concludes that fenpropidin should be **classified as Skin Irritant Category 2**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

One non-GLP study performed on rabbit is available in the CLH report (Anonymous 1979). Several limitations were detected, as the purity of the test material was not reported and animals were observed only for 14 days instead of 21 days. Nevertheless, the DS considered the study to be

compliant with OECD TG 405 and assessed the signs of irritation as unresolved at study termination and relevant for classification.

The CLH report also mentions one report of adverse health effects from 1996 involving 4 workers exposed from the packaging line – general itchiness and smarting of eyes in two workers each was reported (Lorez and Ledgewood, 2003). The DS considered these observations to be related to fenpropidin's well-known irritation potential. These were the only examples of adverse events mentioned in this study.

Based on these observations the DS proposed to classify fenpropidin for serious eye damage, as Eye Dam 1.

One study described in EFSA DAR (2005) was not reported by the CLH report (Cantoreggi et al, 1998). This study has been performed with A-7516 B variant (see "RAC general comments") and has been considered of interest for the current opinion, although minor limitations were detected: animals were observed for 21 days (1 animal) or 28 days (2 animals) post exposure and the age of the animals at the study start was not stated.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The eye damage/eye irritation studies available in the CLH report are described in the table below:

Table: *Summary of the eye damage/eye irritation studies*

Study, guideline, animal strain	**Test substance** **Dose levels, duration of exposure**	**Results**	**Reference**
Acute eye Irritation, Compliant to OECD TG 405, Non-GLP Rabbit New Zealand white 3/group (each sex represented but proportion not mentioned)	Fenpropidin (purity not reported) Single application of 0.1 mL/eye of undiluted test substance Eyes examined after 1 hour and at 1, 2, 3, 7 and 14 days after instillation (Draize scheme).	Signs of irritation in 3/3 rabbits (1–72h post instillation). Mean (24, 48 and 72 h) scores per animal with undiluted test substance: Cornea: 1.3, 1.3, 1.3 Iris: 1.3, 1.3, 1.3 Conjunctivae (redness): 2.7, 3.0, 3.0 Conjunctivae (chemosis): 1.7, 2.0, 2.0 Signs of irritation had not resolved by day 14 (study termination).	Anonymous 1979
Acute eye irritation/corrosion, Compliant to OECD TG 405, GLP Rabbit New Zealand white 3 males	7516 B (purity not reported) Single application of 0.1 mL/eye of undiluted test substance Eyes examined after 1 hour and then at 1, 2, 3, 7, 14, 21 and 28 days after instillation	Signs of irritation in 3/3 rabbits (24–72h post instillation). Mean (24, 48 and 72 h) scores per animal with undiluted test substance: Cornea: 1, 1, 1 Iris: 1, 0, 0 Conjunctivae (redness): 2.0, 2.0, 2.0 Conjunctivae (chemosis): 1.0, 1.0, 1.3 Signs of irritation had resolved by day 28 in all animals (study termination).	Cantoreggi et al, 1998

Following instillation of the undiluted test substance, the study by Anonymous (1979) showed evidence of ocular irritation (see table 2.6.2.5.1-1 of the CLH report for individual scores).

Corneal opacity, iritis (score⩾ 1 all animals), conjunctiva (redness and chemosis, score ⩾ 2 in 2/3 animals) were observed from 1 hour post instillation and persisted until 72h after instillation. Conjunctival redness (all animals), chemosis and corneal opacity (1/3 animal) were still detected after 14 days post instillation. No scores were provided after 14 days. The CLH report states that a test with diluted fenpropidin (3, 10 and 30%) in Neantine was also performed in the same study (no details on solvent was provided in the full study available to RAC). Whereas application of 3 or 10% of fenpropidin caused weak, short-lasting conjunctival redness with no effect on the cornea or iris, application of 30% of fenpropidin induced strong, long-lasting conjunctival irritation, as did the undiluted substance.

In the study by Cantoreggi *et al.* (1998), all animals exposed showed ocular reactions from day one and throughout the 3 days following instillation of the test article in the eye. These reactions consisted of corneal opacity (score 1 during 3 days, all rabbits), hyperaemia of the iris (score 1 during 3 days, one rabbit), conjunctival redness (score 2, during 3 days, all rabbits) and chemosis (score 1 or 2 during 3 days, all rabbits). On day 21, one animal still presented eye irritation (redness of the conjunctiva and corneal vascularisation), whereas all the adverse effects were completely reversible within 28 days.

The Lorez *et al.* (2003) study is a compilation of human medical data collected by Syngenta and is further discussed in the Background Document. It was considered to have doubtful relevance for classification.

Three animals from study by Anonymous (1979) were described as having conjunctival redness, chemosis and/or corneal opacity persisting at least until day 14. As no further observations were made, it cannot be excluded that these reactions would have persisted until day 21. In Cantoreggi *et al.* (1998), one animal had still redness of the conjunctiva on day 21. According to CLP, classification in category 1 is warranted if "*at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days (…)*". None of these studies specified the purity of fenpropidin. Nevertheless, considering the consistency of eye response to fenpropidin exposure, this uncertainty may be less concerning. In addition, fenpropidin is a relatively strong base (pKa=10.1), and skin irritation was induced in acute and chronic studies, which is considered as supportive evidence for classification.

Therefore, in weighing the evidence, RAC supports the DS proposal to **classify fenpropidin as Eye damage 1**.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

According to the DS, although there is evidence of respiratory irritation in single dose and repeated dose inhalation studies in rats, there is no indication of sensitisation. In the Lorez *et al*. (2003) study, respiratory sensitisation was not observed. A fenpropidin-User surveillance study on farmers was performed by Chester *et al.* (1987). No adverse health effect was reported by the representatives in the farmers using the variant A-7516 A (which is identical to the current variant A-7516B, without water). Nevertheless, very few details are available in the studies provided. Therefore, the DS concluded that classification and labelling for respiratory sensitisation is not relevant as no studies are available.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

No animal data are available and all the reported human data are negative regarding respiratory sensitisation (no asthma, rhinitis/conjunctivitis nor alveolitis reported). Nevertheless, important limitations were noted in these studies. No proper respiratory sensitisation tests were performed on farmers in the Chester et al. (1987) study, but only a survey filled by representatives, on only 65 farmers. In addition, bias cannot be excluded (exposure extent, co-exposure, suspicion of misinterpretation of the questions by the representative among other). An anamnesis and physical examination were reported in the Lorez and Ledgewood (2003) study, but again, no appropriate lung function tests were reported. Therefore, RAC considers that although the data did not warrant classification of fenpropidin as respiratory sensitiser, they are not sufficient to make a robust recommendation. Therefore, RAC recommends **fenpropidin does not warrant classification for respiratory sensitisation due to inconclusive data.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Two skin sensitisation GLP compliant tests performed on Guinea Pigs were available in the CLH dossier: one maximisation study (Anonymous 1994c) and one Buehler study (Anonymous 1994b). Both tests were announced to be compliant (although predating) to OECD TG 406, except that the age of the animals was not reported. Both were positive, with erythema in 25% and 40% of the animals (at 24 and 48h, respectively, with intradermal induction of 5% of fenpropidin) in the maximisation study (Anonymous 1994c) and skin reactions in 25% and 45% of the animals (at 24 and 48h, respectively, with topical induction of 60% of fenpropidin) in the Buehler sensitisation study (Anonymous 1994b). Therefore, the DS proposed to classify fenpropidin as a Skin Sensitiser in Category 1B, H317.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The skin sensitisation studies available in the CLH report are described in the table below:

Table: *Summary of the skin sensitisation studies*

Study, guideline, animal strain	Test substance	Dose levels, duration of exposure	Results	Reference
Buehler study, compliant to OECD 406, GLP Guinea pig Pirbright white (Tif: DHP) 10/sex –test group 5/sex – control group	Fenpropidin (purity 97%) Vehicle and control: oleum arachidis	**Induction:** Dermal occlusive application for 6 hours in weeks 1, 2 and 3: 60% fenpropidin **Challenge:** dermal occlusive application	% of animals with positive reactions at 24 and 48 hours: 25% and 45% (respectively)	Anonymous 1994b

Deviation: the age of the animals was not reported.		for 6 hours in week 5 (13-15 days after induction). 30% fenpropidin		
Maximisation study, compliant to OECD 406, GLP Guinea pig: Pirbright white (Tif: DHP) 10/sex –test group 5/sex – control group Deviation: the age of the animals was not reported.	Fenpropidin (purity 97%) Vehicle: oleum arachidis (intradermal) and Vaseline (topical and challenge)	**Induction:** **Intradermal:** 1) FCA / physiological saline (1:1) 2) 5% in oleum arachidis 3) 5% in FCA / physiological saline (1:1) **Topical:** 30% under an occlusive dressing (48h) **Challenge:** 5% under an occlusive dressing (24h)	% of animals with positive reactions at 24 and 48 hours: 25%, 40% (respectively)	Anonymous 1994c
Maximisation study, compliant to OECD 406, GLP Albino guinea pig 2 times 10 females – Groups I and II 2 times 5 females – controls of group I and group II Deviation: the relative humidity of the laboratory was 30-70% instead of 40-70% for technical reasons	A-7516 B, formulated as 750g/L Vehicle: bi-distillated water	**Induction** **Intradermal:** 1) FCA / physiological saline (1:1) 2) 5% in bi-distilled water 3) 5% in FCA / physiological saline (1:1) **Topical:** 100% under an occlusive dressing (48h) **Challenge:** 0,5% under an occlusive dressing (24h)	No positive reaction observed (either at 24 or 48h)	Arcelin *et al.* (2000b)

Preliminary irritation tests (performed on separate animals) revealed no signs of skin irritation after epidermal application of 30% w/v fenpropidin in oleum arachidis; irritation was observed with the undiluted test article and at concentrations of 40%, 50%, 60% and 80% w/v in oleum arachidis. Concentrations of 60% and 30% were therefore used for topical induction and challenge, respectively, in the Buehler sensitisation study performed in guinea pigs (Anonymous 1994b). No positive skin reactions were observed in the negative control animals. No signs of systemic toxicity nor body weight changes following induction with 60% fenpropidin were detected. Challenge exposure caused positive skin reactions in 9/20 test animals after 48h, which corresponds to a sensitisation rate of 45%. In a previous positive control study with 2-mercaptobenzothiazole, 6/20 test animals showed positive skin reactions (sensitisation rate of 30%), which tends to confirm the sensitivity of the test system. No animal deaths were reported.

Preliminary irritation tests revealed good local and systemic tolerability of an intradermal concentration of 5% w/v of fenpropidin in oleum arachidis (concentration chosen based on the solubility of the test article) and no signs of skin irritation after epidermal application at concentrations of 1% and 5% w/v in Vaseline, whereas skin irritation was seen at 10%, 30%, 50% w/v and undiluted fenpropidin (test performed on separate animals). Therefore, a concentration of 5% w/v and concentrations of 30% and 5% were used for intradermal induction,

epidermal induction and challenge, respectively, in the guinea pig Maximisation study (Anonymous 1994c). No positive skin reactions were observed in the negative control animals. No significant skin irritation was reported after induction and there were no signs of systemic toxicity nor body weight changes. Challenge exposure caused positive skin reactions (erythema) in 25% and 40% of the animals 24 and 48 hours after removal of the dressings, respectively. In a previous positive control study with 2-mercaptobenzothiazole, 20/20 test animals exhibited signs of sensitisation, which confirms the sensitivity of the test system. No animal deaths were reported.

Preliminary irritation tests (performed on separate animals) revealed signs of skin irritation after intradermal application of A-7516B at 1%, 3% and 5% w/v in bi-distilled water. Before epidermal application, pretested animals were exposed to intradermal injections of a 1:1 mixture of FCA/physiological saline and were then dermally exposed to fenpropidin. This treatment with FCA might result in a more severe response during pre-test. Mild to moderate skin reactions were seen with concentrations of 1%, 5%, 10%, 15%, 25%, 50% and 75% v/v in bi-distilled water and with the undiluted formulation. No skin reactions were seen at 0.5% v/v A-7516 B. Therefore, concentrations of 5% and 0,5% were used for epidermal induction and challenge, respectively, in the maximisation study from Arcelin *et al.* (2000b). The study was performed in a total of 10 females albino guinea pigs in the control group and 20 females in the test group. The study was conducted in two stages (I and II) such that there were 5 controls for group I, 5 controls for Group II, 10 test-I and 10 test II animals. The two stages of the study followed identical procedures. No positive skin reactions were observed in the negative control animals. No significant skin irritation were reported after induction and challenge after 24 and 48h. One animal of the control group I was found dead on test day 8 (beginning of the epidermal induction procedure) for unknown reasons. Two animals of the test group II were found dead on days 56 and 59. The cause of the deaths could not be established. No other signs of systemic toxicity nor body weight changes were detected. In a previous positive control study with alpha-hexylcinnamaldehyde in PEG 400, 10/10 test animals exhibited signs of sensitisation. Two non-negligible limitations can be reported for this study. First, distilled water was used as the vehicle, which seem to be inappropriate for a hydrophobic substance. In addition, the purity of substance was not mentioned as such, but it was specified that A-7516B is formulated as 750g/l, which is lower than the specification of purity for fenpropidin (960 g/kg, see CLH report). Therefore, less weight was given to this study compared to the study of Anonymous (1994b and c).

No skin sensitisation effects on humans were reported. A classification for sub-category 1B is warranted when $\geqslant$ 30% of the animals inducted with >1% of intradermal dose are considered positive in a Guinea pig maximisation test or $\geqslant$ 15 % of animals inducted with > 20 % topical induction dose are considered positive in Buehler assay. Fenpropidin caused a positive response in 40% of the animals in a GPMT test with an intradermal induction of 5%, and 45% of positive response in a Buehler assay with a topical induction of 60%, which could correspond to skin sensitisation properties with sub-category 1B. Nevertheless, low doses have not been tested to formally exclude a potential for 1A, and according to the CLP guidance: "When Category 1A cannot be excluded, Category 1 should be applied instead of Category 1B." RAC therefore concludes that fenpropidin should be **classified as a Skin Sensitiser in Category 1, H317.**

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

Summary of the Dossier Submitter's proposal

Several repeated dose toxicity studies are available in the CLH report. Under the STOT RE part, 9 studies are described:

- Two oral 28-day range finding studies, one in rats (Anonymous 1994d, compliant with OECD TG 407, GLP) and one in dogs (Anonymous 1993b, no guideline, non GLP).
- Three oral 90-day toxicity studies, two performed on rats (Anonymous 1995a; Anonymous 1981e, both compliant with OECD TG 408 and GLP) and one in mice (Anonymous 1981f, compliant with OECD TG 408, GLP).
- One oral 26-week study on dogs (Anonymous 1981g, compliant with OECD TG 408, GLP).
- One oral 1-year study on dogs (Anonymous 1995b, compliant with OECD TG 452, GLP).
- One 21-day dermal toxicity study performed in rabbits (Anonymous 1981d, compliant with OECD TG 410, non GLP).
- One 28-day inhalation study performed in rats (Anonymous 1981h, compliant with OECD TG 412, non GLP. Purity not reported).

General toxicity was seen in several studies, as indicated by lower body weight relative to controls in rats, mice and dogs. In dogs, vomiting and salivation were also observed (see assessment and comparison with the classification criteria section for more details). In rats, marginal but non-reversible increases in pulmonary foam cells were noted in high dose animals.

According to the DS, the most consistent effect of fenpropidin observed was irritation. This irritation was seen in organs including the oesophagus, stomach and urinary bladder, but also at several locations of the body surface, such as ears and food pads (Anonymous 1981f; Anonymous 1995b) and may be the cause of histological changes observed (epithelium hyperkeratosis and acanthosis in several organs and epithelium urinary bladder hyperplasia). The DS stated that local irritation should not be considered as evidence of specific target organ toxicity.

The liver was identified to be a target organ of fenpropidin, indicated by increased organ weight, hepatocyte hypertrophy and/or increase in alkaline phosphatase activity (Anonymous 1994d and 1995a; Anonymous 1993b and 1995b; Anonymous 1981g). The DS concluded that these findings were consistent with adaptive changes and were not considered to represent severe organ toxicity as defined in CLP guidance. Both Kupffer cell pigmentation and proliferation were seen in dogs in a one-year study (Anonymous 1995b) whereas Kupffer proliferation only was detected in one rat inhalation study (Anonymous 1981h).

The DS concluded that the nervous system was another target of fenpropidin toxicity. Relevant findings consisted of hind-limb paralysis accompanied by demyelination of the spinal cord, which was seen in rats and dogs. Demyelination of different segments of the spinal cord was described in the high dose males of the one-year dog study. One male of this group also showed hind limb paresis (Anonymous 1995b). In rats, one female of the 90-day study (Anonymous 1995a) was found with paralysed hind limbs and demyelination of the spinal cord and peripheral nerves. The cataract seen in both rats and dogs was not considered to represent evidence of significant target organ toxicity by the DS. It was postulated that this effect may be due to an impairment of cholesterol biosynthesis. The DS proposed to classify fenpropidin as STOT-RE Category 2, H373: May cause damage to the nervous system.

An immunotoxicity study (Eapen, 2011) has been conducted with fenpropidin to support regulatory requirements in other regions of the world. This study was performed on mice and in accordance with GLP. Fenpropidin did not significantly suppress the humoral immune response. In addition, a review of the repeated dose toxicity studies mentioned above for fenpropidin was

performed in Bhandal et al. (2016). No evidence of adverse effects on the immune system in rats, mice or dogs was highlighted in the report which therefore concluded that fenpropidin has no immunotoxic potential.

Potential endocrine disrupting properties were also discussed in the CLH report and assessed according to the EFSA-ECHA guidance (2018). Although endocrine disruption is currently not a hazard class under CLP, the information might provide useful information on possible modes of action and might help with the interpretation of the results and is therefore summarised here below.

In a mechanistic study (*in silico* molecular docking approach, described in Devillers et al., 2015), the ability of fenpropidin to bind and act as an agonist/antagonist of androgen receptor (AR), oestrogen receptor α (Erα) or β (Erβ) or thyroid hormone receptor α (TRα) or β (TRβ) was assessed. Overall, the results indicate that fenpropidin has a low theoretical binding potential with oestrogen (α,β) receptors, but may bind to the thyroid (α,β) receptors and may have antagonistic activity on the androgen receptor. The study was assessed by the DS as reliable (with restrictions, see CLH report) but of medium relevance, since it was based on *in silico* data.

Other studies, that could provide valuable information of potential ED properties, were evaluated in the CLH report. The effects seen in the study by Anonymous (2003) seem to be the most relevant. In this study, in addition to reprotoxic effects (described in the Reproductive toxicity section), an increase in incidence and severity of cortical fatty changes in adrenal glands as well as a lymphohistiocytic infiltration of the prostate were mentioned. These effects were attributed to a stress response and/or were considered to be secondary to a depressed bodyweight. The DS concluded that the available data on fenpropidin do not demonstrate effects that would fulfil the criteria for the identification of endocrine disruptors according to the relevant ECHA-EFSA guidance (2018).

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The short-term and long-term toxicity studies (general aspects) available in the CLH report are described in the table below.

***Table**: Summary of the short-term and long-term toxicity studies*

Method, most relevant guideline deviations, if any, species, strain, sex, no/group GV for STOT RE2 classification	**Test substance, route of exposure, dose levels, duration of exposure**	**Results – NOAEL – target tissue/organ – critical effects ***	**Reference**
28-day oral range finding study, compliant with OECD 407, GLP **Deviation:** brain and epididymides weight not recorded, histopathology not performed for spinal cord, no recovery group, no examination of the reticulocytes, sensory activity, grip strength	Fenpropidin technical (purity 97%) Oral (diet) 0, 50, 200, 1000 and 2000 ppm equivalent to 5.40, 20.1, 104.6, 200.1 mg/kg bw/day (males), and 5.62, 19.9, 103.4, 212.2	NOAEL: 1000 ppm (104.6 in males and 103.4 mg/kg bw in females) **200.1 (m) / 212.2 (f) mg/kg bw/day:** *Body weight*: ↓ 28% (m), 14% (f) *Food consumption:* ↓ 27.5% at week 4 (m) *Clinical chemistry:* ↑ urea: 29.5% (m), 25.6% (f); ↑ A/G ratio: 10.1% (m), 11.3% (f); ↑ ASAT: 55.5% (m), 27.1%	Anonymous d

Method, most relevant guideline deviations, if any, species, strain, sex, no/group GV for STOT RE2 classification	Test substance, route of exposure, dose levels, duration of exposure	Results – NOAEL – target tissue/organ – critical effects *	Reference
and motor activity assessment were not performed, additional group for reversibility was not set. Rat: Tif: RAIf (SPF) 5/sex/group **GV: 300 mg/kg bw/d**	mg/kg bw/day (females) Vehicle: Acetone	(f); ↑ ALAT: 122.6% (m), 131.4% (f); ↓ cholesterol: 24.8% (f) *Histopathology:* ↑ non glandular stomach hyperkeratosis: 5/5 (m), 2/5 (f) and acanthosis: 5/5 (m); ↑ esophagus hyperkeratosis: 5/5 (m), 5/5 (f) and acanthosis 5/5 (m); ↑ urinary bladder hyperplasia: 4/5 (m), 4/5 (f) and inflammatory cell infiltration 4/5 (m); ↑ lung alveolus foam cells: 4/5 (m) and 4/5 (f) **104.6 (m) / 103.4 (f) mg/kg bw/day:** *Body weight:* ↓ 10% (m), 7% (f) both not statistically significant *Food consumption:* ↓ 9% (m) over the course of the study *Clinical chemistry*: ↑ urea: 40.0% (m), ↑ ALAT: 83.2% (m) *Histopathology:* ↑ esophagus hyperkeratosis 5/5 (m), 4/5 (f); ↑ lung alveolus foam cells: 1/5 (m), 4/5 (f) **20.1 (m) / 19.9 (f) mg/kg bw/day:** *Body weight:* ↓ 9% (m), 3% (f) both not significant *Food consumption:* ↓ 10% (m) over study course *Histopathology:* ↑ esophagus hyperkeratosis 3/5 (m) **5.40 (m) / 5.62 (f) mg/kg bw/day:** *Body weight:* ↓ 10% (m) *Food consumption:* ↓ 11% (m) over the course of the study	
90-day oral toxicity study, compliant with OECD 408, GLP **Deviations:** Brain, spinal-cord and eyes examined histopathologically only for 5 animal/sex in control and HD group Rat: Tif: RAIf (SPF) 15/sex/group Recovery: 10/sex control and high dose	Fenpropidin technical (purity 97%). Oral (diet) 0, 20, 150 and 1500 ppm equivalent to 1.14, 9.84, 89.9 mg/kg bw/day (males), and 1.24, 10.1, 97.3 mg/kg bw/day (females) Vehicle: Acetone	NOAEL: 150ppm (9.84 and 10.1 mg/kg bw/day for males and females, respectively) **89.9 (m) / 97.3 (f) mg/kg bw/day:** *Clinical observations*: 1/25 females had bilateral opaque eyes and bilateral limb paralysis. *Body weight week 13*: ↓ 16% (m), 8% (f) *Food consumption week 1-13:* ↓ 10% (m), 5% (f) variable significance depending of the week *Water consumption week 13:* ↓ 23% (m) *Haematology:* ↑ WBC 22%, 29%	Anonymous 1995a

Method, most relevant guideline deviations, if any, species, strain, sex, no/group GV for STOT RE2 classification	Test substance, route of exposure, dose levels, duration of exposure	Results – NOAEL – target tissue/organ – critical effects *	Reference
GV: 100 mg/kg bw/d		lymphocytes (f), *Clinical chemistry*: ↓ glucose 12% (m); ↓ triglycerides 29% (m) *Organ weight*: ↑ liver relative weight: 12% (f) *Histopathology*: Nerves demyelination with hind limb paralysis 1/5 (f); ↑ esophagus hyperkeratosis 10/10 (m) and (f), acanthosis 6/10 (m) and 2/10 (f); ↑ non-glandular stomach hyperkeratosis 8/10 (m) and 5/10 (f) – control 1/10 (f), acanthosis 7/10 (m) and 4/10 (f); ↑ urinary bladder hyperplasia 4/10 (m) and 7/10 (f) – control 1/10 (f); ↑ pulmonary foam cells: 9/10 (m) grading 1.7, control 7/10 grade 1.3; 7/10 (f) grade 1.7, control 6/10 grade 1.3. **9.84 (m) / 10.1 (f) mg/kg bw/day:** *Histopathology*: ↑ esophagus hyperkeratosis 4/10 (m and f); ↑ non-glandular stomach hyperkeratosis 3/10 (m) **1.14 (m) / 1.24 (f) mg/kg bw/day:** No treatment related findings	
90-day oral toxicity study, compliant with OECD 408, GLP Deficiencies: Discrepancy of animal age (between m and f), no satellite control, clinical chemistry and tissues examination limited (among other, not performed for spinal cord), no tests performed for neurological endpoints Rat: SPF-albino rats 16/sex/group; satellite group: 6/sex **GV: 100 mg/kg bw/d**	Fenpropidin (purity 94.7%) Oral (diet) 0, 20, 60, 120 mg/kg bw/day Vehicle: distilled mono-glyceride linked to fat globule Satellite group: high dose only, 14 day recovery	NOAEL: 60mg/kg bw **120 mg/kg bw/day** *Clinical observations (as worst, namely, maximum of animals presenting the same symptom in the same time):* scaliness (all animals), hunched posture (13/32), rhagades (17/32), loss of hair (5/12), narrowed palpebral fissures (9/32) and tail necrosis (19/32) *Body weight*: ↓ 30% (m), 13% (f) *Food consumption*: ↓ 18.0% (m), not significant *Clinical chemistry (week 13)*: ↓ Cholinesterase activity: 65% (f); ↓ Cholesterol: 28% (f), 17% (m); ↑ ASAT 14% (m), 47% (f); ↑ ALAT 43% (m), 94% (f) **60 mg/kg bw/day** *Clinical observations (as worst)*: hair loss (4/32), shaggy fur (2/32) and scaliness in tail (1/32) *Body weight*: ↓ 15% (m), 8% (f) *Clinical chemistry (week 13)*: ↓	Anonymous 1981e

Method, most relevant guideline deviations, if any, species, strain, sex, no/group GV for STOT RE2 classification	**Test substance, route of exposure, dose levels, duration of exposure**	**Results – NOAEL – target tissue/organ – critical effects ***	**Reference**
		Cholinesterase activity 41%(f);); ↓ Cholesterol: 21% (f), 20% (m) **20 mg/kg bw/day** *Clinical observations*: hair loss (3/32), shaggy fur (1/62) *Body weight*: ↓ 7% (m), 6% (f)	
90-day oral toxicity study, compliant with OECD 408, GLP Deviations: age at study start not given, no information on randomization, ophthalmological examination only in high dose group and control at week 13, limited parameters for clinical chemistry, spinal cord and nerves (among other) histopathologically not examined, no tests performed for neurological endpoints Mouse SPF-albino 16/sex/group **GV: 100 mg/kg bw/d**	Fenpropidin (purity 99%) Oral (diet) 0, 625, 1250, 2500 and 5000 ppm; equivalent to 0, 58, 155, 359 and 547 mg/kg bw/day (m), and 0, 87, 179, 361 and 566 mg/kg bw/day (f) 625 ppm dose group added following mortality at 5000 ppm	NOAEL: 1250 ppm (155 in males and 179 mg/kg bw in females) **547 (m) / 566 (f) mg/kg bw/day** All animals died or killed for humane reasons. **359 (m) / 361 (f) mg/kg bw/day** *Mortality*: 5/16 f died by week 5, 1 m by week 13 *Clinical signs*: ↑ local skin irritation signs, ear and tail tip inflammation *Body weight*: ↓ 13.8% (m), 10.4% (f) *Clinical chemistry*: ↑ ASAT 100% (m) and 85% (f) *Macropathology*: ↑ Skin hyperkeratosis **155 (m) / 179 (f) mg/kg bw/day** *Clinical signs*: ↑ Inflammation ears and tail tip *Body weight*: ↓ 8.3% (f) **58 (m) / 87 (f) mg/kg bw/day** *Body weight*: ↓ 10.4% (f)	Anonymous 1981f
28-day dose ranging finding study in beagle dog, no guideline, non GLP Deviations: Few animals tested, no recovery group, weightings and histopathology only performed for selected organs (nerves and spinal cord not included) Dog: Beagle 2/sex/group **GV: 300 mg/kg bw/d**	Fenpropidin technical (purity 97%) Oral in capsules 0, 5, 15, 25 mg/kg bw/day 28 days Dose adjusted weekly according to body weight. Controls receive empty capsules	NOAEL: 5mg/kg bw for males and 15 mg/kg bw for females **25 mg/kg bw/day** *Clinical signs*: ↑ vomiting, salivation (1m and 2f) *Body weight*: no significant changes *Food consumption*: ↓ 54.9% week 1 (f) *Clinical chemistry*: ↓ cholesterol 30.9% (m), 26.4% (f); ↑ platelet: 150% (m), 157% (f) *Organ weights*: relative kidney 30% (m); Liver: ↑ relative 59% (m), 20% (f); ↑ absolute 48% (m) **15 mg/kg bw/day** *Clinical signs*: ↑ vomiting, salivation (1f) *Body weight*: no significant changes *Food consumption*: ↓ 62.2% week 1 (f), not significant	Anonymous 1993b

Method, most relevant guideline deviations, if any, species, strain, sex, no/group GV for STOT RE2 classification	Test substance, route of exposure, dose levels, duration of exposure	Results – NOAEL – target tissue/organ – critical effects *	Reference
		Clinical chemistry: ↓ cholesterol 24.4% (m); ↑ platelet: 149% (m) *Organ weight*: ↑ relative kidney weights 17% (m); Liver: ↑ relative weight 38% (m); ↑ absolute 38% (m) **5 mg/kg bw/day** No treatment-related effects	
26-week oral toxicity study in dog (Beagle), compliant with OECD 409, GLP Deviations: two dogs per sex only sacrificed after 26w, ophthalmology after 2 and 26 weeks only, spinal cord histopathology not performed, clinical chemistry not complete, statistical analyses of mean body weights performed for both sexes combined 4/sex/ group, including 2/sex/group after 4 weeks recovery **GV: 49.4 mg/kg bw/d**	Fenpropidin (purity 94.7%) Oral in capsules 0, 2, 5, 12 mg/kg/day 26 weeks, recovery period 4 weeks	NOAEL: 5mg/kg bw **12 mg/kg bw/day** *Mortality*: One male at week 16 *Body weight :* no significant changes at study end *Clinical signs*: Bilateral conjunctivitis, eye keratitis and clouding (1f); ↑ salivation and vomiting (incidence not reported) *Clinical chemistry*: ↑ ALP 93.6% (m), 46.8% (f), ↓ cholesterol (f) weeks 19 and 26, not significant. *Histopathology*: male decedent: hepatitis with congestion and slight cholestasis; enteritis and diapedesis bleeding (relationship to treatment unknown). **5 mg/kg bw/day** *Body weight :* no significant changes at study end Clinical signs: ↑ vomiting (incidence not reported) **2 mg/kg bw/day** No treatment related effects.	Anonymous 1981g
1-year oral toxicity study in dog, OECD 452, GLP Deviations: urine volume and ornithine decarboxylase not measured; femur with joint not taken Dog: Beagle 4/sex/group **GV: 24.6 mg/kg bw/d**	Fenpropidin technical purity (97%) Oral in capsules 0, 2, 5 and 20 mg/kg/day	NOAEL: 5mg/kg bw **20 mg/kg bw/day** *Mortality:* ¼ (m), with hind limb paresis and demyelination of spinal cord *Clinical observations:* ↑ Indurated pads: all animals; vomiting 4/4 (f); scale formation 4/4 (m) and ¾ (f); red skin ¼ (m and f) *Ophthalmoscopy:* ↑ lens opacity: all animals *Clinical chemistry (at study end)*: ↑ AP 115.9% (f); ↓ A/G ratio 32.4% (f); ↑ globulin 33.5% (f); ↓ plasmatic Ca 6.4% (m) *Relative QT interval* increase at weeks 26 and 52 *Organ weight:* ↑ liver relative 27% (m);	Anonymous 1995b

Method, most relevant guideline deviations, if any, species, strain, sex, no/group GV for STOT RE2 classification	Test substance, route of exposure, dose levels, duration of exposure	Results – NOAEL – target tissue/organ – critical effects *	Reference
		↑ liver absolute 31% (m) ↑ kidney relative 26% (f). *Histopathology:* Cataract of crystalline lens (all animals) and demyelination of spinal cord ¾ (m); Epidermis acanthosis ¾ (m), 4/4 (f) and skin/dermis chronic inflammation 2/4 (m), ¾ (f); Hepatocyte hypertrophy (all animals); Kupffer cells pigmentation 4/4 (f) light to high severity – ¼ in control light severity; liver inflammatory cell infiltration ¾ (f) light severity – 2/4 in control light severity; lymphohistiocytic infiltration 4/4 (f) light severity – ¾ in control light to moderate severity; renal tubular pigmentation 4/4 (f) – ¼ in control; urinary bladder epithelium inclusion bodies 4/4 (m), 2/4 (f); lung cholesterol granulomas 4/4 (m), ¼ (f) **5 mg/kg bw/day** Histopathology: hepatocyte hypertrophy 2/4 (m), lymphohistiocytic infiltration 4/4 (f), light severity; Kupffer cell hemosiderosis ¼ (f) light severity **2 mg/kg bw/day** Histopathology : lymphohistiocytic infiltration 4/4 (f), light to medium severity; Kupffer cell hemosiderosis ¼ (f) moderate severity	
21-day dermal toxicity, compliant with OECD 410, Non GLP Deviations: chemical parameters not fully investigated (among others, cholesterol is lacking), spleen not examined, no systemic toxicity at the highest dose, stability in the vehicle not determined Rabbit New Zealand white, 5/sex/group (intact as well as abraded skin were tested)	Fenpropidin (purity 94.7%) Dermal 0, 0.02, 0.2 and 1-2 mg/kg/d Treatment at 2 mg/kg stopped days 10-13 and continued at 1 mg/kg bw/d at day 14-24 6 hours/d (occlusive), 21 days Vehicle aqueous 0.5% CMC	NOAEL: cannot be stated **1-2 mg/kg bw/day** Marked skin irritation with severe fissuring: all animals; oedema: all animals; desquamation: 2/5 (m) and 3/5 (f). Epidermal ulceration, marked epidermal thickening, inflammation of the dermis and occasional dermal fibrosis. Atonia: all animals (m and f) – grading of 1.7 (m) and 1.5 (f) **0.2 mg/kg bw/day** Erythema: 4/4 (m) and 6/6 (f); oedema: 4/4 (m) and 5/6 (f); skin fissuring: 4/4 (m) and 5/6 (f). Epidermal ulceration, marked epidermal thickening, inflammation of the dermis and occasional dermal fibrosis. Atonia: 6/6 (f) – grading of 1.3	Anonymous 1981d

Method, most relevant guideline deviations, if any, species, strain, sex, no/group GV for STOT RE2 classification	Test substance, route of exposure, dose levels, duration of exposure	Results – NOAEL – target tissue/organ – critical effects *	Reference
GV (haber's rule) = 857 mg/kg bw/d		**0.02 mg/kg bw/day** Leucocyte infiltration; Slight skin irritation in most animals (including controls). Atonia not statistically significantly different from control (both occurrence and severity) *In control:* Oedema: 1/5 (m); erythema: 5/5 (m) and 3/5 (f); atonia: 3/5 (m and f) – grading of 1 (m and f)	
28-day inhalation study, compliant with OECD 412, Non GLP Deviations: Treatment of exhaust air not stated, equipment for temperature and humidity measurements not specified, chemical and hematologic parameters not fully investigated (among other cholesterol not investigated), thymus not weighted. Rat: CD (Sprague-Dawley) 15/sex/group; of these 10/sex/group for blood analysis **GV for category 1 (vapor): ≤0.6 mg/l/6h/d**	Fenpropidin (purity not reported) Inhalation (nose only, aerosol droplet) Measured concentration: 0, 20.4, 76.8, 237.4 mg/m3 (equivalent to 0, 0.02, 0.08 and 0.24 mg/l) for 6 hours per day, 7 days per week	NOAEL: cannot be stated. **0,24 mg/l/6h/d:** Nasal passages: resp. 33ersist. Infiltr.: 5/10 (m) and 4/10 (f), resp. epith. Changes: 3/10 (m) and 4/10 (f); Lung, alveolar emphysema: 8/10 – grading 2.9 (m) and 10/10 – grading 2.7 (f) *(controls 3/10 grading 2.3 (m) and 6/10 – grading 2.0 (f))*; Exfoliative dermatitis: 7/10 (m) and 9/10 (f); Thymus lymphoid cell degeneration: 7/10 (m) and 5/10 (f); Liver, Kupffer cell proliferation: 7/10 (m) and 5/10 (f) – No cases in controls *Hematology :* WBC: ↓ 71% (m) and ↓ 76% (f); neutrophils: ↓ 31% (m) and ↓ 48% (f); lymphocytes ↓ 78% (m) and ↓ 80% (f) **0.08 mg/l/6h/d:** Histopathology not performed *Hematology :* WBC ↑ 39% (m); neutrophils: ↑ 147% (m); lymphocytes: ↑ 19% (m) **0.020 mg/l/6h/d** No evidence of systemic toxicity Nasal passages: resp. 33ersist. Infiltr.: 2/10 (f), resp. epith. Changes: 1/10 (m) and 2/10 (f); Exfoliative dermatitis: 1/10 (m) and 1/10 (f)	Anonymous 1981h

* Haematology and chemistry parameters changes: mentioned if ≥ 10% and significant (except if mentioned otherwise), reported body weight changes: at the end of the study. For supplementary data, see CLH report.

Rat oral studies

In Anonymous (1994d) study, rats were exposed to fenpropidin via diet for 28 days. There were no mortalities or treatment-related clinical observations. Bodyweight development was statistically significantly reduced in both sexes of the high dose group. This decrease is not dose dependent and didn't decrease consistently through all exposure levels (see table B.6.3.1.1-1, RAR volume 3, 2021). At week 4, a non-dose dependent but significant decrease of food

consumption was seen in males only. Several liver-related clinical chemistry parameters showed statistically significant variations (see table above).

A statistically significant increase in adrenal (in males and females), testis and thyroid (males) relative weight at the highest dose was detected whereas the absolute organ weights were within the expected range. Therefore, this observation may be a consequence of the decreased body weight and was not considered treatment related. The variation of kidney absolute and relative weight was inconsistent between sexes and doses and thus was considered to be of unclear relevance.

In addition, there was a dose related increase irritation signs in several organs (hyperkeratosis and acanthosis in stomach and esophagus, hyperplasia and inflammatory cell infiltration in the urinary bladder, no occurrences in control) seen from 20 mg/kg bw/day in the oesophagus and an increased incidence of foam cells in the lung alveoli detected in both sexes at doses slightly exceeding the guidance value of 100 mg/kg bw/day (1/5 in control, both sexes). The prothrombin times increased dose dependently and this increase was statistically significant for males of all treatment groups and in females of the highest dose group (31.83, 36.61*, 39.92**, 39.50**, 40.12** sec and 29.30, 26.77, 30.39, 31.92, 34.37** sec at 0, 50, 200, 1000 and 2000 ppm for males and females respectively (* $p<0.05$ and ** $p<0.01$).

No mortality was observed, also in the 90 day study by Anonymous (1995a). There was a statistically significant decrease in body weight in males and females at the highest dose. The food consumption was reduced in males and was statistically significant throughout the study (except on week 13), whereas decreased food consumption was statistically significant only on weeks 1, 2 and 6 in females. Haematological and chemical parameters revealed a tendency towards lymphocytosis in females (statistically significant at the highest dose) and a decrease in plasma triglyceride and glucose levels, also statistically significant, in males. Consistent with the 28 day study of Anonymous (1994d), there were general signs of multi-organ irritation (in the esophagus and non-glandular stomach). Erosion or ulceration and inflammatory cell infiltration of the non-glandular stomach were recorded at the high dose as well as diffuse hyperplasia of the urinary bladder epithelium, together with an increased incidence of inflammation in both males and females. No occurrences were detected in respective controls (except if noted otherwise in the table above). As also observed in the 28 day study by Anonymous (1994d), there was an increase in alveolar foam cells in the lung in both sexes of high doses groups (irreversible in males), but in contrast to the 28 day study this finding was also seen in the control group.

Neurological effects: One female exposed to 97.3 mg/kg bw/day had bilateral opaque eyes (day 56) and bilateral hindlimb paralysis (day 76). According to the authors, the latter finding correlated with histopathologically diagnosed nerve demyelination (spinal cord, cranial and spinal nerve roots and proximal peripheral nerve) and was considered treatment-related, as such changes are not common in rats of that age and since no occurrences were detected in controls. In each group, no other observations that covered the functional domains of CNS activity, CNS excitation, sensorimotor, autonomic and physiological functions were detected.

In the 90 day study by Anonymous (1981e), no mortalities were observed. Body weights were dose dependent and statistically significantly reduced in all treated animals. A decrease of food consumption was seen in males, but this was not statistically significant. No macroscopic abnormalities were observed. There was no evidence of any effect of treatment on organ weights or on histopathological findings, but data regarding histopathological findings were only provided for the control and HD groups. Several clinical observations were described in all exposed groups, including, among others rhagades and scaliness that can be linked to the irritative properties of fenpropidin. Although loss of hair was also observed in control females and females treated at

20 and 60 mg/kg, the effects described in the high dose group appeared to be more severe (tail necrosis, narrowed palpebral fissures).

A statistically significant increase of ALT and AST was described at the highest dose in males and females. At study termination, a statistically significant decrease in cholinesterase activity was observed at mid and high dose in females, whereas a statistically significant decrease of cholesterol was observed in both males and females from mid and high dose group (-20 and -17% in males and -21 and -28% in females from mid and high dose groups, respectively). The author of the study also stated that alveolar cells occurred in alveolar spaces of rats treated with 120 mg/kg more frequently than in those of control rats.

Mouse oral study

There were some limitations in the 90 day study by Anonymous (1981f), leading to the DS to consider this study as supportive only. All mice died or were killed for humane reasons at 547-566 mg/kg bw/d, having presented signs of general toxicity. The clinical signs observed were considered to be related to the irritative potential of fenpropidin. Therefore, a new group (58-87 mg/kg bw/day) was introduced 7 weeks after study initiation. A statistically significant decrease in body weight was detected in all dose groups (although non-dose dependent). No remarkable changes in haematological parameters, organ weight, macroscopic or histological findings were described (except hyperkeratosis and changes or loss of PAS positive cytoplasmic granules in 2500 ppm dose animals), but clinical pathology and histopathology examinations were limited, among other cholesterol was not measured and the spinal cord and peripherical nerve were not examined (see RAR 2021, volume 3). High doses groups (5000, 2500 ppm and 1250) shows dose-related irritability and inflammation of the nose, ears and/or tail tip and toes (resulting in necrosis at the highest doses).

Dog oral study

The Anonymous (1993b) 28 days oral dog study was considered as supportive only by the DS due to the low number of dogs tested (2/sex/group). No mortalities or significant changes in body weights occurred, although statistically significant decreases in food consumption were seen in females in the highest dose group during week 1 and 2 (-54,9% and -40%, respectively). Clinical signs at week 1 were vomiting in one 15 mg/kg group female, one male and two females (pronounced) from the 25 mg/kg male dose group, with salivation. The eye examination revealed no treatment-related signs. Microscopic examination was not performed on the spinal cord.

A statistically significant increase in platelets was observed at 15 (males) and 25 mg/kg bw/d (males and females), in addition to a statistically significant decrease in cholesterol. Statistically significant increases in liver weight (absolute and relative) and kidney weight (relative) were detected in males from 15 and 25 mg/kg bw/day. Mottled lungs were reported in both exposed and control animals (one 15 mg/kg male, two 25 mg/kg males, two control females and two 15 mg/kg females) and therefore appeared to be of unknown toxicological relevance at least in females. According to the authors, at microscopic evaluation these changes were related to chronic inflammatory changes observed in the lung of all animals on study, and therefore were not considered treatment related.

The 27 weeks oral dog study by Anonymous (1981g) was only considered as supportive by the DS due to the number of deviations compared to the respective OECD guideline (see RAR volume 3, 2021 for list of deviations). Most relevant deviations are included in the table above). One high dose group male died on week 16 following a steady weight loss from week 11 onwards. This animal had stomatitis, enlarged thyroids (not evaluated histopathologically), enteritis and hyperemia in the stomach and intestines. Microscopic findings in the liver and intestines were also found (hepatitis with congestion and slight cholestasis; enteritis and diapedesis bleeding). The real cause of death remains unclear (loss only 6% of its initial weight, lesions found were

estimated to be of slight severity), nevertheless, according to the authors, hepatitis might have been the result of treatment with fenpropidin, as one dog of the pilot study exposed to 100 mg/kg also shows elevated liver enzymes and hepatitis. No statistically significant body weight changes were seen in other animals at the end of the study.

Vomiting (with salivation in some females) occurred after capsule administration in all exposed groups with an increasing frequency at 25mg/kg, although the incidences were not reported. One female from the high dose group also showed conjunctivitis and keratitis in both eyes (detected at week 20), followed by a clouding of the lenses. A statistically significant increase of ALAP in males and females from the highest dose group (week 26 and 30) was also seen. No additional macroscopic or microscopic findings were found.

In the one-year oral dog study by Anonymous (1995b), no spontaneous mortality was mentioned. Only transient food consumption and body weight decrease were observed, in females and at the very beginning of the study (weeks 1 and 4, see CLH report). One animal was sacrificed following clinical signs of paresis, whitish appearance of both eyes, reduced food intake, body weight loss. Microscopy investigation showed that this animal had also marked demyelination of the thoracic spinal cord segments. No statistically significant body weight changes were observed at study termination in other animals. Signs of irritation (indurated and inelastic pads, scale formation, reddening of the skin), confirmed histologically (epidermis acanthosis and inflammation), were seen in all animals of the 20 mg/kg bw/day dose group (both males and females, see table above), whereas no respective occurrences were observed in controls or the two lower dose groups.

Vomiting occurred in all exposed animals and was more severe in females of the 20 mg/kg dose group. In addition to transient changes in platelet and albumin plasma levels (males), some significant changes in AP activity and globulins levels were described in the high dose group (females). Consistently, an increase in relative and absolute liver weight were described as well as histological findings in animals of the 20 mg/kg bw/day dose group (especially females), with few occurrences of hepatocyte hypertrophy and pigmentation of Kupffer cells in control (see the table above). Nevertheless, the incidence and severity of lympho-histiocytic infiltration in the control group was rather high (see table above and table B.6.3.2-22 from the RAR, volume 3, 2021). An increase in relative kidney weights was statistically significant in females of the 20 mg/kg/day dose group and was observed along with the presence of pigmentation of renal tubular cells, inclusion bodies in the urinary bladder epithelium, with minimal to moderate hyperplasia, with no or low occurrence in respective controls (see table above).

A slight, but statistically significant decrease in blood calcium in males from the highest dose group was also detected (6%, dose dependent at week 52). The relationship with the treatment was assessed as unlikely by the DS, as the calcium level was low during the pretest period as well.

All males (included the sacrificed one) and one female from the high dose groups had minimal to moderate cholesterol granulomas consisting of cholesterol crystals surrounded by histiocytes in the lung parenchyma. According to the author, the granulomas accounted for the mottling observed macroscopically. Nevertheless, granulomas weren't reported in every group where mottling of the lungs was detected (see table B.6.3.2-22 of the RAR vol 3, 2021) therefore the link appears to be uncertain.

Neurological effects: All males and females from the high dose group showed opacity of the lenses (first signs detected at week 22), and the males were also affected by bilateral cataracts of the eyes ranging from moderate to marked and correlated to the macroscopic diagnosis of whitish eyes. In addition, ¾ males from this group also had demyelination of the spinal cord. None of these effects were seen in control animals. The finding of a focal to multifocal necrosis of skeletal muscle fibers in males, increasing in severity in high dose, was considered by the

author to be the result of the decreased motility (which was not recorded), secondary to the cataract and/or the demyelination in the spinal cord. Nevertheless, the occurrences of this finding are dose dependent (0, 1, 2 and 3 males affected at 0, 2, 5 and 20 mg/kg/d, respectively), and are already seen at doses below those where cataracts and demyelination were reported.

Electrocardiography revealed a statistically significant prolongation of the relative QT-interval (QTc) for high dose males at weeks 26 and 52 (in the full study: QTc mean week 26: 191.086 in controls compared to 202.169 at the high dose, p=0.012; QTc mean week 52: 193.476 in controls compared to 210.261 at the high dose, p=0.001, p(trend)=0.006)). The QTc increase at week 26 was considered as incidental due to the absence of a dose-response relationship. The QTc increase at week 52 is not discussed in the study, and the authors instead comment that visual comparison of the configuration of the electrocardiographic tracings (not provided) with the pre-test pattern revealed no reaction to treatment. Therefore, the extent of concern is difficult to evaluate with the level of detail provided.

Rabbit dermal study

In Anonymous (1981d), rabbits were exposed 6h per day to fenpropidin dermally. Three control animals, one animal from the 0.02 mg/kg/day dose group and two animals exposed to 0.2 mg/kg/day died or were killed for humane reasons during the study. In a majority of cases, the animals showed loss of appetite and no significant *post-mortem* finding were described. Apart from this mortality, no systemic toxicity was found. There were no significant differences in skin irritation response between abraded and intact skin, in any group (data reported in table above were obtained from intact skin group). Due to the marked skin irritation observed in the high dose group (2 mg/kg bw/day), treatment was abandoned on days 10-13 and continued at 1 mg/kg bw/day on day 14 for 10 further days.

Signs of skin irritation occurred in all treatment groups, including controls (see table above) but effects were more severe in the exposed group (RAR vol 3, 2021, Table B.6.3.3-1). Oedema, desquamation and fissuring were seen at mid and high dose only. Slight skin irritation was confirmed in the control by microscopic examination as mild acanthosis and slight dermal leukocyte infiltration were described. At the low dose of 0.02 mg/kg/day, a marginal increase in leukocyte infiltration was seen and at the mid dose of 0.2 mg/kg/day and above, epidermal ulceration, marked epithelial thickening, inflammation of the dermis and occasional dermal fibrosis were also apparent. No other treatment-related effects were described. Due to the highly irritative nature of the test-substance, testing of higher doses was precluded and no systemic toxicity was achieved.

Rat inhalation study

In Anonymous (1981h), rats were exposed to fenpropidin via inhalation (nose only). In 5/15 controls (8 deaths in total in control), 6/15 low dose, 4/15 mid dose and 5/15 high dose rats deaths were attributed to suffocation in the restraining tubes (see full study report) and were therefore considered unrelated to the treatment. In the high dose group, four other females were found dead in their cage, whereas two died during the exposure period (between treatment days 6 and 7). Animals from the mid dose group presented hyperactivity and respiratory distress with wheezing and more severe condition was seen in females (hunched posture). In the high dose-group, the clinical signs were similar but more severe, with tremor observed in addition in females. Therefore, all animals in the mid and top dose were sacrificed after the first week of treatment due to their poor condition. No statistically significant changes of body weight nor food consumption were reported for control and low dose groups.

Variations in WBC (including neutrophils and leucocytes) appear inconsistent (see table above), with an increase at the mid dose (both sexes, statistically significant in males) followed by a significant decrease in the high dose group (both sexes). Statistically significant changes in

several chemical parameters (see table B.6.3.3-7 from the RAR 2021, vol 3) were also seen, which most of the time consisted of notable increases/decreases at the high dose only, just before death, after 2 weeks of exposure. No significant changes were seen at the low dose, except a GOT increase of 19% in males at week 4, and a very slight decrease of Na+ (3%), which appear inconsistent with the increase at the highest doses. These adverse events seem more related to acute toxicity and may not be relevant for STOT RE classification. This observation also applies to the increase of RBC in males from the high dose group. No significant organ weight changes were reported in the mid dose group (males and females) whereas the weights (relative to brain) of liver, gonads, kidney and spleen were statistically significantly reduced in males from the high dose group. In high dose group females, a statistically significant decrease of spleen weight (relative to brain) and a statistically significant increase of adrenals weight (relative to brain) were also mentioned (see RAR 2021, volume 3, table B.6.3.3-8).

In the RAR, volume 3 (2021), histopathological findings were described at the highest dose (see table B.6.3.3-9). Among them, signs of irritation (inflammatory infiltration and epithelial changes) in the nasal passages were mentioned. Three cases of respiratory epithelial change were also reported at low dose (one in male, two in females) and two cases of respiratory inflammatory infiltration are reported in low dose females (no histopathological results available for mid dose). No occurrence was mentioned in either male or female controls. Exfoliative dermatitis was detected in both males and females from high and low dose group, with dose dependency and was not mentioned in control animals. Pneumonitis and perivascular cell infiltration in the lung were often seen in control animals, whereas a dose dependent increase in incidence of alveolar emphysema (with an increase in severity grading in the high dose group compared to controls, see table above) was noted. Kupffer cell proliferation in liver and lymphoid cell degeneration in thymus were reported in high dose animals only.

Other studies

Anonymous (1983) is an 80 weeks mice oral (dietary) combined carcinogenicity and toxicity study (GV: 16 mg/kg bw/d, see Carcinogenicity part for more details). The purity of fenpropidin was not stated. The survival fell below 50% in males only during the 3 last weeks of the study. A decrease in body weight of more than 10% was observed in males from high dose group only on week 80. The only clinical signs reported at doses warranting classification consisted of hyperkeratosis of the esophagus. No changes in organ weights at doses warranting classification were recorded.

A developmental neurotoxicity study in Wistar Han rats was performed by Anonymous (2011a). In this study, animals were exposed to fenpropidin via the diet from gestation day 6 to lactation day 21. An irritative effect was detected with an increased incidence of scabbing and hair loss around the mouth and forelimbs in dams of the 400ppm group, which was attributed to treatment.

A rat dietary two-generation reproductive toxicology study was performed by Anonymous (2003). The thymus weight tends to decrease in most generations (pups and adult, except in F1), with increased presence of phagocytic cells in cortex and increased incidence of cortex atrophy in F1 and F2 pups. Nevertheless, these changes were not seen in adults, and the relative thymus weight was statistically significantly increased in F1 adults (in high dose males and females). Some inconsistencies in the changes in liver weight were seen between F0, F1 and F2 generations (see RAR 2021, volume 3). In F1 and F2 pups liver, a decrease in glycogen deposition and extramedullary haematopoiesis (incidences and/or severity) were observed at the two highest doses, whereas the liver lymphocytic infiltration decreased in F0 and F1 adults. Other additional histopathological changes were seen, including a decrease in spleen extramedullar hematopoiesis in high dose F1 and F2 pups as well as cortical fatty changes in adrenals of F0 and F1 adult females.

Abstract and relevance of the main effects observed for STOT RE classification:

- Neurotoxicity: Adverse effects on the nervous system were seen in both rat and dog after fenpropidin exposure. In the 90 days oral rat study (Anonymous 1995a), one female presented bilateral hind limb paralysis in the presence of spinal cord and peripheral nerve demyelination at doses that warrant classification as STOT RE 2 (i.e. 97.3 mg/kg bw/day). As this effect is not common in animals of this age, and not seen in controls, this finding was considered to be relevant for classification. The spinal cord was histopathologically examined in 5 animal/sex only (control and HD group), therefore it cannot be excluded that effects might have been detected at lower doses if more animal had been examined. In addition, demyelination of the spinal cord was also seen in 75% of the male dogs in a 1 year oral study (Anonymous 1995b), again at a dose warranting classification as STOT RE 2 (applying Haber's rule results for an exposure dose of 20 mg/kg bw/day). One male of this group also showed hind limb paresis. Effects on the spinal cord were not reported in any other study, but among these negative studies, the spinal cord was histopathologically examined only in the study performed by Anonymous (1994d). No effects were seen in this 28 day study, that may indicate that a longer exposure duration is needed (90 days) to induce these neurotoxic effects. Tremors were seen after acute oral exposure to fenpropidin (Anonymous, 1981b; Arcelin *et al.*, 2000a), and could also be indicative of neurotoxic effects.
  The observed effects, i.e. demyelination of spinal cord and peripheral nerves, in some instances associated with hind limb paralysis in a considerable number of animals exposed at doses relevant for STOT RE 2 are considered to represent significant adverse effects. RAC therefore concurs with the DS that fenpropidin should be classified as STOT RE2, with the nervous system designated as the target organ.

- Eye effect: Adverse effects on the eye were observed in both rats and dogs after oral exposure to fenpropidin. In the study by Anonymous (1995b) one year exposure resulted in the formation of lens opacity in all dogs (males and females) at the top dose of 20 mg/kg bw/day, which is relevant for classification as STOT RE 2. After 26 weeks of fenpropidin exposure, clouding of the lenses were described in one female dog at a dose relevant for classification as STOT RE2 (i.e 12 mg/kg bw/day) in the Anonymous (1981g). Several deviations were reported in this study (see table above and the RAR, 2021), nevertheless, RAC is of the opinion that the eye effect reported should not be dismissed, as consistent with what has been seen in the Anonymous (1995b) study, and still consider this finding as supportive. No relevant eye defect was described in dogs after shorter exposure (28 day study from Anonymous 1993b). Bilateral opaque eyes was also seen in one female rat at a dose relevant for classification as STOT RE 2 (i.e 97.3 mg/kg bw/d), after 90 days of exposure (Anonymous 1995a). No relevant eye defect was described in two other rat studies (28 day study by Anonymous 1994d and another 90 day study by Anonymous 1981e) nor in a 90 day oral mouse study (Anonymous 1981f), which could indicate that some species and strains might be more sensitive to these effects and that the time of exposure could have a role in the appearance of this adverse effect.
  The DS postulated in the CLH report that both the cataract formation and nerve demyelination may be linked to impairment of cholesterol biosynthesis. It is noted that cholesterol levels were decreased in the studies by Anonymous (1994d), Anonymous (1993b), Anonymous (1981e) and Anonymous (1981g), which is in support of the DS proposal. Nevertheless, no further details on this potential mode of action were presented in the CLH report, that could demonstrate a link between possible interference with cholesterol synthesis and effects on eyes or the nervous system. RAC is of the opinion that no firm conclusion can be drawn on the relevance of this mode of action.

Cataract formation and eye clouding/lens opacity are considered by RAC to be significant adverse effects. This effect was clearly seen in one species (dog) in the study of the longest duration (1 year, all animals affected in Anonymous 1995b), at doses relevant for STOT RE 2). Support comes from single incidences in a dog exposed for 26 weeks and a rat that was exposed for 90 days. These observations were made at doses relevant for classification as STOT RE 2 via the oral route (capsule and diet). The available studies via the dermal and inhalation route did not show eye effects, but were of shorter duration than the studies resulting in eye effects. In conclusion RAC proposes to classify fenpropidin as STOT RE 2, eye.

- Irritation: Irritation was widely observed in most of the studies, with hyperkeratosis of several organs (esophagus, stomach), acanthosis, hair loss, scaliness. These effects are considered to be long lasting irritative effects caused by the irritant nature of the substance, which resulted in the proposal to classify fenpropidin as Skin Irrit 2, STOT SE 3, H335 and Eye Dam 1 and are not considered supportive for classification as STOT RE.

- Liver effects: A dose dependent increase (statistically significant in highest dose) in relative liver weight was seen in females from one 90-day rat study (Anonymous 1995a), but not in the other rat studies (only a decrease of relative liver weight was described in top dose males in Anonymous 1981h study). This weight increase was also detected in dogs after 28 days and 1 year exposure (Anonymous 1993b; Anonymous 1995b, respectively), but not after 26 weeks (Anonymous 1981g). In the 1 year dog study there was also a dose dependent increase in the incidence of hepatocellular hypertrophy. While these effects could indicate an adaptive response of the liver, some studies also mention other signs of liver toxicity. Hepatitis with congestion and slight cholestasis was seen in a decedent male dog from the 26 week dog study (Anonymous 1981g), and the study authors stated that this effect might have been favored by the administration of fenpropidin. Also, increased incidences of Kupffer cells pigmentation and liver inflammatory cell infiltration were seen in the 1 year dog study at dose that could warrant classification STOT RE 2 (see table above, Anonymous 1995b). Nevertheless, this finding has to be considered in relation to the incidences in the controls (i.e. Kupffer cell pigmentation; ¼ control females, inflammatory cell infiltration: 2/4 control females). These effects were not seen in the other studies (only Kupffer cell proliferation in Anonymous 1981h). Blood biochemical alterations indicative of liver damage were seen in rats and dogs including increases in liver enzymes ASAT and ALAT (see table above). An increase in ASAT activity was also seen in the 90 day mouse study (Anonymous 1981f). These effects were not correlated with histopathological changes. In addition, a decrease in cholesterol levels was reported in rats and dogs (Anonymous 1994d; Anonymous 1993b; Anonymous 1981e and, non-significantly in Anonymous 1981g) as well as a decrease of triglyceride levels in the 90 day rat study by Anonymous (1995a). Although that seems to point to an effect on lipid metabolism, evidence that would enable the liver to be identified as the direct cause of this effect was not provided. The adversity of this finding alone, without sufficient mechanism of action information to link it with an alteration in any organ/tissue function or morphology, seems uncertain. Overall, RAC considers the observed effects on liver to not be sufficiently consistent between studies to support a classification of fenpropidin as STOT RE for liver effects.

- Nasal passages: A clear dose-related increase in incidence and severity of local irritation in the nasal passages was seen in the 28 day inhalation study performed on rats (Anonymous 1981h), with inflammation and changes in olfactory and respiratory

epithelium after fenpropidin exposure. There were no occurrences of this finding in the controls.

Table : *28-day inhalation study in rats with fenpropidin: incidence (grading) of nasal passages findings (Anonymous1981h).*

Dose (mg/l/6h/d)	Males				Females			
	0	**0,02**	**0.08**	**0.24**	**0**	**0,02**	**0.08**	**0.24**
olfact. 41ersist. Infiltr.	0/10	0/10	-	1/10 (2.0)	0/10	0/10	-	2/10 (1.5)
resp. 41ersist. Infiltr.	0/10	0/10	-	5/10	0/10	2/10 (1.5)	-	4/10 (1.8)
olfact. Epith. Changes	0/10	0/10	-	0/10	0/10	0/10	-	2/10
resp. epith. Changes	0/10	1/10	-	3/10	0/10	2/10	-	4/10
Mortality	4/15	3/15	3/15	2/15	4/15	3/15	1/15	9/15

This effect could be of concern, although this study had several limitations (among other high levels of mortality including, in controls, purity and batch of fenpropidin not mentioned, treatment of exhaust air not stated and equipment for temperature or humidity measurements not specified). In mid and low doses groups, the deaths were attributed to suffocation due to turning round in the restraining tubes and not to exposure to the test article (as described in the full study report). Epithelium changes and inflammatory infiltration in nasal passages occurs rapidly, as detected at the highest dose, in animals sacrificed after the first week of exposure. In addition, signs of respiratory distress at the mid and high dose group were already described after one single exposure (more severe in females), which is considered to be an acute effect. The first occurrences of respiratory epithelial changes are also described at the low dose (0.02 mg/l/6h/d) in males and females at study termination. In females of this group, respiratory inflammatory infiltration was also described (see table above). Histopathology was performed after 28 days in surviving animals from the control and low dose groups and should therefore not be considered as acute effects. Altogether, a classification STOT RE 1 could be warranted. Nevertheless, the CLP guidance states that "*Where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure. In such a case classification with STOT-SE only would be appropriate.*" Therefore, these results are considered supportive for classification STOT SE3 (respiratory irritation) proposed above.

- Lung: An increase in pulmonary foam cells was seen in rats and dogs. In a 28 day oral rat study (Anonymous 1994d), lung alveolus foam cells were seen from doses below the guidance value for STOT RE 2 classification (i.e 103.4 mg/kg bw/day) in 4/5 females, while the incidence in respective controls was low (1/5). In a 90 day oral rat study (Anonymous 1995a), most males and females from the high dose group (i.e 89.9 mg/kg bw/day in males and 97.3 mg/kg bw/day in females) showed increases in pulmonary foam cells at doses that warrant classification as STOT RE 2. Nevertheless, the occurrence in control in this study was high (7/10 and 6/10 for males and females, respectively) with a similar grading (1.3 in control compared to 1.7 in exposed animals), raising doubt about

toxicological relevance of this result. Anonymous (1981e) described small areas with interstitial inflammatory infiltration and alveolar macrophages in the 120 mg/kg/bw/d dose group, therefore at doses too high to be relevant for classification for STOT RE. No histological data were provided for the lower doses. In a 1 year dog study (Anonymous 1995b) a slight increase of mottled lung was observed in males (from the mid dose of 5 mg/kg bw/d with ¼ male at the mid dose and 2/4 at the high dose, none in controls) and 4/4 males had lung cholesterol granuloma (at the high dose of 20 mg/kg bw/d, none in controls). The effects were seen at doses relevant for classification as STOT RE 2. No or uncertain cases of inflammatory infiltration or cholesterol deposits were described in shorter studies in dogs and mice. A difference in sensitivity between strains cannot be excluded.
While the impairment of function appears uncertain, these findings have an impact on organ morphology and therefore are considered relevant for STOT RE classification (see Annex I: 3.9.1.3 of the CLP guidance: "*These adverse health effects include consistent (…), or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ,(…)*".

- Immunosuppressive effect: Atrophy of phagocytic cells in the thymus seen in Anonymous (2003) might indicate an immunosuppressive effect of fenpropidin. This effect is noted in pups and therefore was induced by exposure during *in utero* development. Therefore, this finding is not relevant for STOT RE classification. In F0 adults, only decreases in the thymus absolute weight in females were noted. In addition, even with uncertainties, Eapen *et al.* (2011) seem to indicate the lack of relevant effect of fenpropidin on humoral immune response. In addition to these results, lymphocytosis was detected at a dose that warrants classification as STOT RE 2 in female rats (i.e. 97.3 mg/kg bw/day, Anonymous 1995a), which however appears not fully consistent with the increase of lymphocytes at 76.8 mg/m3 in males rats followed by a major decrease at the high dose in the Anonymous (1981h) study. Therefore, the eventual properties on immune system remains inconclusive to RAC.

RAC concurs with the DS and supports a classification as STOT RE 2 for neurotoxicity. In addition, RAC is of the view that a classification as STOT RE 2 is also warranted for effects on the eye and lung. Based on the toxicokinetic information on fenpropidin it cannot be excluded that neurotoxicity and eye effects would be induced via routes other than the oral route.

In conclusion, RAC proposes to **classify fenpropidin as STOT RE 2 (nervous system, eye, lung)**, without specifying a route of exposure.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Fenpropidin has been tested in several *in vitro* tests and in one *in vivo* genotoxicity assay:

- In Hertner et al. (1993a), a reverse mutation test was performed in bacteria. This study predates but was considered compliant with OECD TG 471 (with deviations: the number of cells per culture was not stated), and GLP. The purity of fenpropidin was of 97%.
- A gene mutation assay on Chinese hamster lung fibroblasts was described in Strobel *et al.* (1988). This study predates - but was considered compliant with - OECD TG 476 (with deviations: source of cells was not given, results on mycoplasma testing was not presented, the required level of survival was not achieved for the maximum dose tested

and 2-acetyl aminofluorene was used as a positive control instead of 3-Methylcholanthrene, Dimethylbenzanthracene or Benzo[a]pyrene) and GLP. The purity of fenpropidin was of 91%.

- Hertner *et al.* (1993b) described an *in vitro* chromosome aberration test in Chinese hamster ovary cells. This study predates but was considered compliant with OECD TG 473 (with deviations: less than 300 well-spread metaphases were scored; a short-term treatment in the absence of S9 mix was not performed, mitotic index was used for cytotoxicity assessment instead of recommended parameters and percentage of solvent in final medium not indicated) and GLP. The purity of fenpropidin was of 97%.
- The same laboratory also performed an *in vitro* UDS assay (Hertner *et al.*, 1993c). This study was considered compliant with OECD TG 482 and GLP. The purity of fenpropidin was of 97%.
- Hertner *et al.* (1993d) also described an *in vivo* mouse bone marrow micronucleus test. This study was considered compliant with OECD TG 474 (with deviations, which included, among others: micronuclei were evaluated at 2 doses only due to mortality, animal body weights at study start only given as a range, mean and standard deviations not given for micronucleate erythrocytes per group, only 4 of 5 male animals were evaluated at mid dose due to death, 1000 immature erythrocytes were counted per animal only) and GLP. The purity of fenpropidin was of 97%.

All the results were negative, with and without S9 (when applicable). Positive as well as negative controls were valid (when mentioned), according to the DS. Therefore, the dossier submitter did not propose classification for germ cell mutagenicity.

Genotoxicity studies on groundwater metabolite CGA289267 have been performed. These studies have not been previously submitted for EU review. All three genotoxicity studies (Woods *et al.*, 2017; Gilby *et al.* 2017 and Gilby *et al.* 2017, for further details, please refer to the CLH report) met the requirements and gave clearly negative responses, therefore CGA289267 was not considered to be toxicologically relevant.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The *in vitro* and *in vivo* genotoxicity studies available in the CLH report are summarised in the table below.

***Table**: Summary of the short-term and long-term toxicity studies*

Test method		Results	Reference
Ames test TA98, TA100, TA1535, TA1537 and WP2uvrA	0, 31.25, 62.5, 125, 250 and 500 µg/plate (original) 0, 125, 250, 500, 1000 and 2000 µg/plate (confirmatory) Vehicle: acetone ± S9	Negative. Cytotoxicity occurred at concentrations of 1000 and 2000 µg/plate in all strains	Hertner *et al.* 1993a
***In vitro* gene mutation assay** V79 cells (Chinese hamster lung fibroblasts	- S9: 0, 10, 60, 70, 80, 90 µg/ml (exp. 1-3) + S9: 0, 5, 20, 30, 40, 50, 60, 70 µg/ml (5-50 µg/mL for	Negative Relative survival was reduced to 10-58% and 8%, with and without S9	Strobel *et al.* 1988

	experience 1 and 20-70 µg/mL for experience 2). Vehicle: DMSO	(respectively), after treatment (80 or 60 µg/mL)	
***In vitro* chromosome aberration test** Chinese hamster ovary cells	0, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250 and 500 µg/mL ± S9 (original) 3.91, 7.81, 15.63, 31.25 µg/ml without S9 15.63, 31.25, 62.5 µg/ml with S9 recovery (confirmatory) Vehicle: acetone	Negative Higher concentrations than 15.63 µg/ml (-S9) or 31.25 µg/ml (+S9) revealed an insufficient number of scorable metaphases due to toxicity (mitotic index suppressed by more than 80% compared to controls). Final concentration higher than 62.5 µg/ml of culture medium could not be scored due to cytotoxicity and formation of precipitates	Hertner *et al.* 1993b
***In vitro* UDS assay** Males rat hepatocytes freshly isolated	0, 0.49, 0.98, 1.95, 3.91, 7.81, 15.63 µg/mL (original) 0, 0.48 , 0.97, 1.94, 3.88, 7.75, 15.5 and 31 µg/mL (confirmatory) Vehicle: acetone	Negative for concentrations with at least 25% of viable cells (i.e. ≤ 15.6 µg/ml)	Hertner *et al.* 1993c
***In vivo* bone marrow micronucleus test** Tif: MAGF (SPF) mouse 5/sex/group	0, 385, 770 or 1540 mg/kg bw (gavage) Vehicle: carboxymethyl cellulose (CMC) 0.5%	Negative Mortality was seen between 1920 and 5000 mg/kg bw in pretests, but still high during testing at 1540 mg/kg bw	Hertner *et al.* 1993d

In vitro assays

In the Hertner *et al.* (1993a) study, five strains of bacteria were tested with and without S9 (derived from rats). As no toxicity was observed at the highest concentration tested in the original experiment, the concentration range for the confirmatory experiment was increased to a maximum concentration of 2000 µg/plate. No precipitates or aggregates were detected. No statistical analysis was performed. Positive controls were chosen by strain, and except for the strains TA98 and TA1537 (without metabolic activation, 20 and 150 µg/plate, respectively) as well as TA1535 and WP2 uvrA (with metabolic activation, 400 and 50 µg/plate, respectively), the concentration tested for these PC were between 2 and 5 µg/plate. Based on the fact that the number of revertant colonies was more than ten times higher in the positive control than in the negative control and in fenpropidin exposed cells (which were comparable), RAC considers that no indications that fenpropidin could induce gene mutation in bacteria was provided.

Chinese hamster lung fibroblasts (V69 cells) were pre incubated in growth medium for 24 hours and then exposed to fenpropidin for either 16 hours (without metabolizing system) or 5 hours (with metabolizing system derived from male albino mice) in a gene mutation test (Strobel *et al.*, 1988). The cytotoxicity of fenpropidin was determined by measuring cell viability shortly after the exposure time (day 2), from which relative cell viability was derived considering the viability of the solvent control as 100%. Cloning efficiency was also assessed as a measure of cytotoxicity (at day 7). Mutant frequencies were calculated as the number of mutant colonies per 10^6 cells. The number of HGPRT-mutant cells in the treated groups did not exceed the spontaneous background level, in the presence or absence of metabolic activation (see table 2.6.4.1-4 of the

CLH report). A marginally but statistically significantly higher mutant frequency was seen at 70 µg/ml in the second experiment in the absence of S9 (see table 2.6.4.1-3 from the CLH report), without a dose-response relationship. This result was not reproducible. Therefore, this effect could be incidental. The 2AAF used as positive control significantly induced HGPRT-mutant cells, according to the author of the study.

In the Hertner *et al.* (1993b) chromosome aberration test, three experiments were performed on Chinese hamster ovary cells (CHO). In the first one, the cells were exposed during 18h to fenpropidin in the absence of S9. In the second one, cells were exposed during 3 hours to fenpropidin, followed by a recovery period of 21 hours, in the presence of metabolizing system (derived from the rat). Whenever possible 200 metaphase spreads (100 per replicates) were examined. During the confirmatory study, these two experiences were repeated, in addition to two others: one where cells were exposed to 42h of treatment without S9 and another where cells were exposed to 3h of treatment with metabolic activation followed by 39 hours of recovery. None of these values showed a statistically significant difference when compared with their respective negative control. When available, the positive control was considered suitable (no positive control for the two lasts experiments). Altogether, although limitations were identified in the study, there is no indications that fenpropidin could induce chromosome aberration *in vitro*.

The same laboratory also performed an autoradiographic DNA repair test on rat hepatocyte *in vitro* (Hertner *et al.*, 1993c). The cells were exposed to fenpropidin for 16 to 18 hours. Statistical analysis was not performed, except means and standard deviations were reported for triplicates of each treatment and control groups. The test has been considered to be positive by the authors if (1), the mean (gross and net) number of silver grains per nucleus show a concentration increase of minimum two compared to control and with at least one of the mean net value which was ≥ 2.0 and (2), the percentage distribution of the (gross and net) number of silver grains show an obvious shift to higher values in at least two or more subsequent concentrations compared to their respective control distribution. The authors reported that the increase seen in exposed cells was insufficient to meet the criteria for a positive response. In addition, the slightly higher NNG values observed for several concentrations in the confirmatory experiment were not reproducible. Positive and negative controls were valid. Therefore, these results do not bring evidence of specific DNA damage after fenpropidin exposure in the rat primary hepatocytes *in vitro*.

In vivo assay

One *in vivo* bone marrow micronucleus test was performed in mice by Hertner *et al.* (1993d). The mice were given one dose of the test substance via gavage and bone marrows were extracted 16, 24 and 48 hours post dosing. The doses were selected based on a primary tolerability test where mortality was seen between 1920 and 5000 mg/kg bw. Nevertheless, a high mortality rate was still detected at the highest dose (1540 mg/kg bw) and therefore only two doses could be evaluated. Furthermore, one male died in the intermediate dose group, leaving only 4 of 5 males to be evaluated. The low dose group and positive control were only assessed for micronuclei at 24 hours post treatment, whereas the mid dose group wasn't evaluated 48h post treatment. For each animal the ratio of polychromatic to normochromatic erythrocytes was determined and 1000 polychromatic erythrocytes were scored for micronuclei. In all treated groups as well as in the positive controls the ratio of polychromatic to normochromatic erythrocytes was not statistically significantly different from the negative controls and therefore gave no indication for bone marrow toxicity. With one exception, no statistically significant increase of micronucleated polychromatic erythrocytes (mnPCE) percentage was observed in the treated groups. The marginally but significantly higher mnPCEs percentage (males and females pooled) at 24 hours seen in the low dose group did not meet the criteria for a positive response for the author as the value obtained was 0.11%, which is below the maximum of the range accepted for negative

control (0.20%). Positive and negative controls were valid. Therefore, although several limitations are observed in this study, there is no evidence of a clastogenic or aneugenic potential detected in the performed *in vivo* study of bone marrow micronuclei.

No toxicokinetic data on mice was available in the dossier. In rats, fenpropidin is rapidly absorbed after one oral dose and excreted within 48h. The absorbed dose is distributed mainly between liver and kidney. While the residue is still high in liver and kidney 24h after oral application (13.938 ppm and 5.087 ppm respectively, see table B.6.1.1-3 of the RAR 2021, volume 3), the residue in bone (femur) was one of the lowest in all the organs tested, and was 0.798 ppm after 24h. This could raise some doubt on the relevance of using bone marrow as a target organ for genotoxicity after fenpropidin exposure, especially as it was not demonstrated that the bone marrow was reached in the *in vivo* micronucleus test. In addition, *in vitro* the metabolism seems much higher in rats than in humans (Sayer *et al.*, 2017).

Overall, RAC notes some limitations in the genotoxicity data but the available *in vitro* studies cover bacterial, yeast and mammalian gene mutations, chromosomal aberrations, and DNA repair. No concern has been identified as all tests were concluded negative. An uncertainty relates to the *in vivo* test on micronuclei formation. While the study was concluded negative, convincing evidence for target organ exposure was not demonstrated either in this study nor in other relevant studies. Moreover, TK data suggested low systemic bioavailability following extensive liver first pass effect and rapid excretion. Nevertheless, as the *in vitro* studies where negative for both chromosomal aberrations and mutagenicity, RAC agrees with the DS that **fenpropidin does not warrant classification for germ cell mutagenicity based on the available data**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two oral long-term toxicity/carcinogenicity studies in rats and mice were described in the CLH report.

The first study is a GLP EPA 83-1 2-year chronic toxicity/carcinogenicity study in rat (Anonymous 1988). This study was considered compliant with OECD TG 453, but with limitations: due to toxicity in the highest dose group (absolute body weight reductions of > 10% and irritative properties in both males and females), doses were revised after 7 weeks of treatment. The low and mid doses were lowered to 2 and 10 ppm respectively. The two highest dose-level groups were given control diet for 4 weeks and then changed to 50 and 250 ppm until termination of the study.

Non-significant increases in pancreatic islet cell adenomas were reported in male rats at the top dose. This incidence was within the historical control data (HCD) range. In addition, the DS highlighted that pancreatic islet cell adenomas were not increased at the interim sacrifice compared to controls. Therefore, the effect was concluded to be of spontaneous origin related to the aging of the rats.

The second study is a mouse carcinogenicity study (Anonymous 1983). This study was considered compliant with OECD TG 451 and 453, but with limitations, including, among others: the purity was not mentioned, the survival of the high dose-group males was lower than 50% at study termination, the individual clinical signs were recorded but not presented in the report, the statistical analysis only comprised means and standard deviations (except for mortality), blood sampling, clinical chemistry and haematology examinations were limited, adrenals were not weighted and uterus, rectum, sternum and bone marrow were not preserved. No treatment-related neoplastic findings were reported at any dose level in mice.

Based on the results of two oral long-term toxicity/carcinogenicity studies in rats and mice, DS concluded that fenpropidin was not carcinogenic in either species and therefore proposed no classification.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The long-term toxicity and carcinogenicity studies available in the CLH report are described in the table below.

***Table**: Summary of the long-term toxicity and carcinogenicity studies*

Method, most relevant guideline deviations, if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results – NOAEL – target tissue/organ – critical effects*	Reference
2-year chronic toxicity/ carcinogenicity study, EPA 83-1, compliant with OECD 453, GLP **Deviations:** dose level changed after 7 weeks, survival below 50% at study termination in most groups. Rat: CD-Crl: CD (SD) BR 70/sex/group except high dose 80 sex/group 10/sex/group interim kill (12 months) 10/sex/group for clinical pathology (20/sex for high dose group)	Fenpropidin (purity 91%). 0, 5/2, 25/10, 125/50, 625/250 ppm (diet). Equivalent to (2d dosage) : 0.07, 0.34, 1.68 and 8.53 mg/kg bw/day (males) and 0.09, 0.45, 2.27 and 11.83 mg/kg bw/day (females) Vehicle: acetone	NOAEL (systemic): 50 ppm: 2.27 mg/kg bw/day (f) based on decrease of absolute bodyweight, no systemic adverse effect on males From week 96, survival in males fall below 50% in all but 10/25 ppm dose group. In females, survival fall below 50% at study termination in 10/25 and 50/125 ppm dose group. **Non-neoplastic findings 625/250 ppm** *Clinical signs:* Signs of local irritation until dose decreased and then reappears later in study. *Body weight*: No significant changes at end of study *Body weight gain:* ↓ 14% (f) week 11-80 *Clinical chemistry:* ↑ potassium concentration 14.3% males and 15.6% females. **Doses between 125/50 and 625/250 ppm (included):** *Hematology:* Significant changes but without dose dependency: ↑ Hb: 13.2 – 17,4% males *Organ weight*: ↑ relative pituitary weight: 38 – 44% (no dose-response) **Doses between 25/10 and 625/250 ppm (included):** *Hematology:* Significant changes but without dose dependency: ↑ red cell parameters (RBC and PCV) 13.2 – 21% males *Organ weight*: ↓ absolute ovary weight: 24 – 73% (no dose-response) **2/5 ppm :** No significant effect **Neoplastic findings** No significant increase	Anonymous 1988
Carcinogenicity study, 80 weeks, compliant with	Fenpropidin (purity not reported)	NOAEL (systemic): 300 ppm (41.9 mg/ kg bw/day in males and 51.7 mg/kg bw/day in females)	Anonymous 1983

OECD 451/453, GLP **Deviations:** survival below 50% at terminaison in high dose group males, no statistical analysis except mean and standard deviation, not all organs, clinical chemistry and hematology parameters were evaluated Mouse: Crl: Crl:CD-1 (ICR)BR 63/sex/group	0, 30, 100, 300 and 1000 ppm (diet) Equivalent to 0, 4.12, 13.54, 41.90, 143.8 mg/kg bw/day (males) and 0, 5.47, 17.70, 51.71, 166.1 mg/kg bw/day (females) Vehicle: corn oil	**Non-neoplastic findings** **1000 ppm** *Mortality:* 45% survival until week 80 (m); 51% survival until week 65-76 (m), > 71% in all other groups including control *Body weight:* ↓ 11% m, 7% f *Food consumption*: ↓ 12% f *Clinical signs:* ↑ incidence of local irritation on forepaws, ears and tail (dermatitis, hyperkeratosis). *Pathology:* Mandibular lymph node hyperplasia: 12/51 (f) – control 3/51; ↑ irritation of GI tract (hyperkeratosis of esophagus and forestomach: 49/51 and 27/51 in males, respectively; 48/51 and 39/51 in females. *In control, no occurrence except stomach hyperkeratosis in f: 4/51*) **Doses up to 300 ppm (included):** No treatment-related findings **Neoplastic findings** **1000ppm:** No treatment-related findings	

*Body weight: at the end of the study

In a 2-year chronic toxicity and carcinogenicity study, fenpropidin was administered in the diet to 70 (or 80 for the high dose group) male and female rats per dose group (Anonymous 1988). Groups of 10 (20 for the high dose group) animals per sex and group were used for clinical pathology examinations. After 12 months, 10 animals/sex/group were selected for interim kill.

The doses were revised after 7 weeks of treatment due to extensive toxicity in the high dose group, namely strong clinical signs of skin irritation as well as slight decreases in body weight and body weight gain compared to controls (-12 and 18% bw; -31 and 37% bwg on week 7 in females and males, respectively) associated with a significant reduction in food consumption. These irritation signs consisted of dry and flaky skin around the mouth and forepaws, hind feet and tail and sore skin around the mouth. In addition, a substantial number of animals also showed sore skin at the forepaws, scabs on the tail and dark discoloured tail tips. Some rats demonstrated a slight lack of grooming, ulcerations of the tail and missing tail tips. Recovery occurred during the four week recovery period. Whereas no changes in body weight was noted at study end (after dose revising), low incidences of dry and flaky skin were detected in high dose animals at later stages of the study.

Survival was less than 50% for all male groups and for the 10 and 50 ppm female groups (see table below). After 96 weeks, survival was close to or below 50% for all male groups, including controls, with the exception of the highest dose-group (62%). According to the OECD guidance document 116 (supporting test guidelines 451, 452, and 453), "*For a negative result to be acceptable in a rat carcinogenicity bioassay, survival in the study should ideally be no less than 50% in all groups at 24 months (…)*". Therefore, this very low survival, especially in controls and in the lowest dose group (thus, *a priori* not linked to fenpropidin exposure) raises questions on the acceptability of this study.

***Table**: Survival (%) in a 2-year chronic toxicity and carcinogenicity rat study with fenpropidin (Anonymous 1989)*

ppm	Males					Females				
	0	**2 (5)**	**10 (25)**	**50 (125)**	**250 (625)**	**0**	**2 (5)**	**10 (25)**	**50 (125)**	**250 (625)**
Week 52[1]	100	92	100	92	98	98	100	98	100	98
Week 78[1]	86	76	82	80	86	92	94	78	90	86
Week 96[1]	46	46	52	46	62	64	72	54	66	64
Week 104[1]	30	36	38	28	50	54	52	40	50	56
Termination[1]	30	32	36	28	48	52	50	40	48	56
Week 96[2]	45	48	52	48	60	62	70	55	65	64

1 carcinogenicity group animals only
2 all animals

At the terminal sacrifice, significantly lower relative pituitary weights were noted in 50 and 250 ppm females but without dose-response nor association with histopathological findings. Absolute ovary weights were significantly increased in females from 10 to 250 ppm compared to controls, but no dose-response relationship was seen. An increased incidence of ovary cysts (5/70, 7/70, 12/70, 12/70 and 9/80 for 0, 2, 10, 50 and 250 ppm, respectively) and follicular cysts (11/70, 6/70, 14/70, 12/70 and 11/79 for 0, 2, 10, 50 and 250 ppm, respectively) were reported, without a dose-response relationship. No correlation between low pituitary weight and high ovary weight at any dose were noted. According to the RAR (2021, volume 3), the notifier provided historical data for the terminal pituitary relative to body weight values to show that the control group was outside the HCD range, whereas the treatment groups were within the HCD range. They also provided HCD to show that the absolute ovary weights were within the HCD range for all groups. Altogether, these findings seem of low relevance for carcinogenicity classification.

On week 103, statistically significant increase in several red cell parameters (RBC, Hb and PCV) were described in mid to high dose males (see table "Summary of the long-term toxicity and carcinogenicity studies", above), but without dose dependency and without consistency over the treatment period. A statistically significant increase in potassium concentration in high dose males and females compared to controls was also described.

Whereas no clearly increased incidences of pancreas islet cell carcinoma were reported compared to controls groups (see the CLH report), an increase in pancreatic islet cell adenomas (23%, compared to 8.6% in control group) was reported in male rats at the top dose of 8.53 mg/kg bw/d (see table below). This increase was not statistically significant (Fisher's exact test).

***Table**: Occurrence of neoplastic pancreatic lesions in rats in a 2-year chronic toxicity and carcinogenicity feeding study (Anonymous 1989 – extract from the CLH report)**

Dose (ppm)	**0**	**2 (5)**	**10 (25)**	**50 (125)**	**250 (625)**
Pancreas islet cell adenomas in males					
Interim sacrifice	0/10	-	-	-	0/10
Terminal sacrifice	2/17	5/19	4/21	4/17	10/32
Unscheduled deaths	4/43	3/41	3/39	4/43	8/38
Total (%)	**6 (8.6)**	**8 (13)**	**7 (12)**	**8 (13)**	**18 (23)**
Pancreas islet cell adenomas in females					
Interim sacrifice	0/10	-	-	-	0/9
Terminal sacrifice	6/30	6/31	4/25	1/30	4/40
Unscheduled deaths	4/30	3/29	2/35	0/30	0/30
Total (%)	**10 (14)**	**9 (15)**	**6 (10)**	**1 (1.7)**	**4 (5.1)**

* Data from the neoplastic lesion table (carcinogenicity animals) + incidences from result tables from interim sacrifice and clinical pathology animals of groups 1 and 5

The available HCD for the performing laboratory were compiled from studies conducted +/- 5 years of the date of the in-life phase of the 2-year rat study in rats of a similar age and strain (period Jan 1981-Dec 1992). The HCD for pancreas islet cell adenoma in male rats indicated a mean incidence of 13.9% with a range between 0% and 54.0% (See the CLH report).

Overall, increased incidences in pancreatic islet cell adenomas were noted in all male rat treated-groups compared to the control-group. However, this observation was limited to one sex, in rats only, the increase was non-significant and non-dose dependent. In addition, β-cell adenomas are benign neoplasms, which lowers the concern, and no occurrences of these adenomas were found at the interim sacrifice, which tends to indicate that the tumour latency remains unchanged compared to the control. Finally, the analysis of HCD shows that β-cell adenoma is a common neoplastic effect in male rats from this strain. For the above-mentioned reasons (benign, late occurrence, not statistically significant, one sex), RAC is of the opinion that this neoplastic finding is not relevant for classification.

In the Anonymous (1983) combined carcinogenicity and toxicity study, fenpropidin (purity unknown) was administered via diet to male and female Crl: Crl:CD-1 (ICR)BR mice (63 mice per dose group). The survival fell below 50% for high dose-group males ($p<0.05$) during the 3 last weeks of the study. No single cause was identified but the incidence of urogenital disease was marginally increased.

Clinical signs consisted mainly of signs of local irritation, including dry flaky skin on the tail, forepaws and ears in high dose females (RAR table B.6.5-11). Bodyweights were slightly decreased in high dose males and females (unknown statistical significance, see table " Summary of the long-term toxicity and carcinogenicity studies", above). Pathology revealed a slight increase in relative liver weight (13%) in high dose males and in the relative kidney weight of females (11%) at the interim sacrifice. There were no treatment-related effects on the organ weights at the terminal sacrifice. As the marginal increases in relative liver and kidney weight were not accompanied by any other sign of a treatment-related effect in these organs (clinical pathology parameters or histopathological evaluations), they are considered not to be adverse. No histological findings were reported for these organs. Local irritation was also observed during histopathology analysis with dermatitis of the skin and hyperkeratosis of the oesophagus and forestomach in high dose animals (RAR table B.6.5-11).

In the RAR (2021, volume 3), a slight increase in mandibular lymph node hyperplasia was reported in females from high-dose groups (Table B.6.5-11). A potential secondary response in the dermatitis was suggested. In the same table in the RAR (2021, volume 3), an increase could be detected in male mice as well, without any dose response relationship, with even higher occurrences at 300 ppm (4/51, 3/16, 7/15, 1/14, 9/51 at 0, 30, 100, 300, and 1000 ppm, respectively) than in females from the high dose group (12/51). Therefore, a background effect unrelated to fenpropidin exposure could not be excluded either. The incidence of lymphoma was not affected by treatment. There was no treatment-related neoplastic finding reported in any group.

RAC notes than both studies had serious limitations. Among others, the survival was below 50% in minimum one group at study termination. Another study, with lower doses designed to retain a sufficient survival rate at the end of the study could have been useful.

In conclusion, relevant findings after exposure to fenpropidin were limited to non-significant increases in the islet cell adenomas in male rats. These tumours were only observed in a single species and one sex, are considered benign and of late occurrence. RAC therefore considers that **classification for carcinogenicity is not warranted.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

The DS presented two dietary two-generation studies in rat, which were conducted according to OECD TG 416 or equivalent and GLP.

The DS reported that the study by Anonymous (2003) was conducted according to the most recent OECD 416 TGD (2001), with minor deviations (some sperm parameters and tissue analyses were not conducted and the post-lactation ovary was not examined) and concluded that these deviations were unlikely to have an impact on the conclusions drawn. The DS considered the second study (Anonymous 1987) as a supplementary only as it had major deficiencies, most prominent of these being the lack of systemic toxicity due to low dosing, since up to the maximum dose of 100 ppm (m: 8.02 mg/kg bw/day; f: 9.31 mg/kg bw/day) no parental toxicity, reproductive toxicity or offspring toxicity was observed.

The focus of the assessment is therefore on the study by Anonymous (2003). Results of both studies are summarised in the table Relevant information on the available two-generation studies, section "Assessment and comparison with the classification criteria".

The DS summarised the relevant observations made by Anonymous (2003) at the top dose of 80 mg/kg bw as a decrease in body weight gain in females during pre-mating, gestation and lactation, for F2 litters a reduction in the number of uterine implantations, pups delivered and live born and for both the F1 and F2 litters and a decrease in body weight gain starting from the lactation period reaching up to 36%. The DS concluded that the decrease in pup body weight was the cause of the observed delay in puberty in males and females. Several differences in organ weights and microscopic findings were also ascribed to the observed body weight decrement. The DS described that there were comparable effects at the next lower dose of 42 mg/kg bw/day, except that there were no effects on number of live born pups and that the body weight development of the pups was only affected from day 4 of lactation.

The DS concluded that there was no direct effect on the development of the offspring, all treatment related changes in the offspring were attributable to systemic toxicity in the parental generations and did not propose to classify fenpropidin for adverse effects on sexual function and fertility.

Developmental Toxicity

The DS summarized the results from four developmental toxicity studies, two in rats (Anonymous 1994e; Anonymous 1981i) and two in rabbits (Anonymous 2011b; Anonymous 1981j).

The available developmental neurotoxicity study in rats conducted in accordance with OECD TG 426 (Anonymous 2011a) was described and considered by the DS in the STOT RE section only.

The DS reported that three of the developmental toxicity studies (Anonymous 1994e; Anonymous 1981i; Anonymous 1981j) had deviations from the OECD TG 414 (2001) or pre-dated it and that the gestational exposure duration was shorter than recommended in the current test guideline.

However, the DS considered the rat study by Anonymous (1994e) was adequate and relevant to assess the potential of fenpropidin to induce developmental toxicity in the rat (despite the shorter exposure duration), whereas they concluded that Anonymous (1981i) & Anonymous (1981j) had major deviations from the test guideline and considered them as supplementary only (see table Overview on developmental toxicity studies). The purity of fenpropidin was not reported for these studies and Anonymous (1981j) was not performed according to GLP (though it followed

scientifically accepted standards). The second developmental toxicity study in rabbits (Anonymous 2011b) was fully compliant with the test guideline and GLP, and therefore considered by the DS as reliable and adequate to conclude on fenpropidin's potential to induce developmental toxicity in rabbits. The results of the studies are summarized in the table Overview on developmental toxicity studies", section "Assessment and comparison with the classification criteria".

The DS concluded that in the key oral (gavage) rat study by Anonymous (1994e) maternal toxicity was seen at the top dose (reduced body weight (not significant), reduced food consumption) without consequential reduction in fetal body weight. The DS considered the observed malformations detected in one fetus in control as well as in one fetus at the top dose as incidental, and not related to treatment. No other relevant findings were reported.

The DS described considerable deficiencies for the second oral (diet) rat study (Anonymous 1981i), i.e. lack of full necropsy of maternal animals, use of only 7 litters per group for visceral examination and 10 litters for skeletal examination and test material was of unknown purity. The DS did not report that this study also included a group of animals which was exposed up until weaning (for further details on the study see section on "Assessment and comparison with the classification criteria"). In contrast to the gavage study by Anonymous (1994e), Anonymous (1981i) reported some maternal toxicity (effects on body weight gain – partly compensated after treatment between days 17 and 21 – and food consumption) already at lower (dietary) exposure levels. The DS did not consider the observed decrease in fetal body weight as adverse, as it was not dose dependent and only considered the increase in incised neural arches at the top dose, where also split, poorly ossified and/or half present neural arches were observed as adverse (see tables Overview on developmental toxicity studies, and Foetal examination of the rat developmental toxicity study by Anonymous (1981i)). Incised neural arches were increased with dose, starting at the lowest dose tested and were reported to be inside HCD in the low dose, but outside the HCD in mid and top dose (though the actual HCD were not presented).

In both rabbit studies maternal toxicity was also observed, as indicated by body weight loss between day 7 and day 20 in the top dose of the Anonymous (1981j) study and by reduced body weight gain and food consumption in the top dose of the study by Anonymous (2011b), at comparable gavage doses.

The DS concluded that the study by Anonymous (1981j) was severely compromised as there were non-treatment related deaths in all dose groups, which were described to be caused by application errors (3 for the low dose, 1 for the mid dose and 1 for the top dose) and broken vertebral columns (1 dam each for mid and top dose) or were of unknown cause (1 each in control and mid dose, 2 each at the low dose and top dose) and food consumption was not recorded. The DS concluded that it was likely that the difference in fetal body weight was attributable to the difference in litter size. Multiple defects were noted in one fetus each of the low dose and of the top dose groups, but were considered spontaneous, as they were said to be observed frequently in this strain (though no HCD were presented). The findings in the 5 mg/kg animals: omphalocele (liver and intestine), missing left ear, missing tail, rudimentary eyelid and maxilla, aplastic left forepaw, multiple skeletal anomalies and in the 30 mg/kg animals: ectopia (liver, stomach, intestine), missing tail, torsion of left hind paw at the knee-joint, skeletal anomalies. No other effects were observed in this study. The DS considered the study as supplementary only.

The DS considered the study by Anonymous (2011b) as the key rabbit study. In the top dose increased incidence of severely malaligned sternebra(e) as well as persistent truncus arteriosus was observed.

The DS described the findings as outside the HCD for malaligned sternebra(e), but just within the HCD for persistent truncus arteriosus (see tables Overview on developmental toxicity studies, and Incidences of malformation in the rabbit study by Anonymous (2011b)). The latter effect was still considered relevant by the DS as it was in the upper range of the HCD (the mean: 0.1%, median: 0%, 75th quartile: 0%; exact incidences are listed in tables Overview on developmental toxicity studies, and Incidences of malformation in the rabbit study by Anonymous (2011b)). The DS further noted that in a statement from the applicant it was mentioned that the upper range of the HCD was influenced by findings in a single litter with only 2 viable fetuses (i.e. 50% incidence). The DS considered the HCD for persistent truncus arteriosus additionally provided by the applicant as not relevant. This information consisted of two studies, in one study there were 4 fetuses of 4 litters with persistent truncus arteriosus, 3 in the low dose, 1 in the mid dose, and in the other study 3 fetuses of 3 litters with this effect were seen at the mid dose. The DS noted that in none of these studies persistent truncus arteriosus was seen in the control group and he therefore questioned the usefulness of these data as historical controls.

The DS also mentions the applicant's statement on the second type of developmental effect, i.e. severely malaligned sternebrae. In this statement the applicant considers the incidence of 3 fetuses in 3 litters in the top dose group was more consistent with a spontaneous event rather than an effect of treatment in the absence of other skeletal effects. The DS does not present his view on this statement. For more details see section "Assessment and comparison with the classification criteria".

The DS also reported that the total number of fetuses with malformation increased with dose and that it was significantly increased compared to controls in the top dose group. For this conclusion the cases of severely malaligned sternebrae were included (otherwise not statistically significant), although the DS was of the view that this effect had been downgraded from malformation to variation (referring to the website devtox.org) and listed the effect in table 2.6.6.2-4 of the CLH report as a variation. RAC notes that under this link severely malaligned sternebrae are not listed as variations, but as belonging in the grey zone of effects between a malformation and variation.

Overall the DS summarised the findings in rabbits by Anonymous (2011b); i.e. increased incidence of severely malaligned sternebra(e) and persistent truncus arteriosus in the top dose) and the observations in rat by Anonymous (1981i); i.e. dose dependent increase in number of incised neural arches, with an increased number of split, poorly ossified and/or half present neural arches at the top dose) as relevant effects, but mentions the poor quality of the study by Anonymous (1981i) and concludes that this study was not adequate for classification purposes and should be considered as supplementary only.

Overall the DS concluded that classification as Repr. 1B was not supported, but that despite some uncertainties there was still sufficient evidence to support classification in category 2.

Lactation

The DS summarised that there were three studies potentially relevant for the assessment of adverse effects on or via lactation, the two two-generation studies (Anonymous, 2003; Anonymous 1987), as well as the developmental toxicity study (Anonymous 1981i), which included a littering phase (Sub-group II). The studies are described in detail in the sections on "sexual function and fertility" and "developmental toxicity" under "Assessment and comparison with the classification criteria" (Table Relevant information on the available two-generation studies, below).

The DS concluded that the observed systemic parental toxicity at 500 & 1000 ppm was the cause of the impaired pup growth (i.e. hampered weight gain). The DS further concluded that although pup growth was affected from day 0 at 1000 ppm and from day 4 at 500 ppm in the Anonymous

(2003) study, there was no indication of decreased pup viability during lactation. A body weight loss of 12.5 g was noted in F0 high doses females (days 0-21) whereas a gain of 18.8 g was detected in control animals for the same period, and the body weight gain was 93% lower in F1 females than in controls. Despite these significant effects on the lactating females, the DS concluded that the quality of the milk and the ability of the mothers to nurse their young were not impaired. The results of a rat developmental toxicity study (Anonymous 1981i) with a littering phase did not provide evidence of an adverse effect on lactation due to fenpropidin at dose levels up to 87.8 mg/kg bw/day.

Therefore, the DS concluded that there were no effects observed that would warrant a classification of fenpropidin for effects on or via lactation.

Comments received during consultation

Industry submitted comments on the hazard class developmental toxicity consisting of two comprehensive documents.

One document was signed by the study author of Anonymous (2011b). The document focused on the occurrence of severely malaligned sternebrae in this study, and stated that the cases seen at the top dose, i.e. in 3 foetuses of 3 litters, was only slightly above the highest incidence in the HCD (August 2006 – November 2014), where 2 foetuses in 2 litters were affected.

RAC agrees that the incidence in 3 foetuses in 3 litters is not high and notes that it only just exceeds the maximum observed incidence of 2 foetuses in 2 litters seen in one study of the HCD data, however, it is noted that in the vast majority of HCD not a single case of severely malaligned sternebrae was seen, as can be easily concluded from the HCD overview provided in the CLH report (no incidence in 127 studies, single incidences in 15 studies and 2 foetuses in 2 litters in two studies).

The document further explains the difference between slightly / moderately malaligned sternebrae versus severely malaligned sternebrae and describes that when the study report of Anonymous (2011b) was prepared severely malaligned sternebrae were classified as a malformation. They further describe that in the 3 cases of severely malaligned sternebrae in the study, there was no indication that alignment of the coastal cartilage was adversely affected (indicating that this finding had little impact on this region of the skeleton). They further state that in 2018 the justification to classify severely malaligned sternebrae as malformation was reevaluated in the conducting laboratory and it was concluded that all severities of this effect should be classified as developmental variation, however, without referring to any reference for this conclusion.

The DS agreed with the reclassification of severely malaligned sternebrae from malformation to variation and in the CLP report referred to the website devtox.org to support this statement. However, RAC noted that under this link severely malaligned sternebrae are listed as a grey zone effect, indicating that devtox.org considers these effects to be in between a malformation and a variation. RAC further notes that malaligned sternebrae in humans was linked to scoliosis (Kenanidis *et al.*, 2018) which is considered to be a severe condition and demonstrates one of the characteristics of malformations, i.e. "*a structural anomaly that alters general body*

conformity". RAC further notes that in the ECETOC monograph No. 031[2] (2002) severely malaligned sternebrae are referred to as malformations.

The second document was prepared by a consultant (TCI) and evaluated the observed incidences of persistent truncus arteriosus and severely malaligned sternebrae in Anonymous (2011b) in relation to the other PNDT studies (one rabbit study and two rat studies).

They considered the 3 incidences of persistent *truncus arteriosus* in the top dose as true malformations but provided further data to be considered as HCD. On one hand these data consisted of two studies (Case study 1 & 2) in which incidences of persistent truncus arteriosus was seen in the lower dose groups (i.e. 3 foetuses in 3 litters in the low dose and 3 foetuses of 3 litters in the mid dose, respectively). However, in line with the DS, RAC considers them not to be adequate as HCD, as treated groups cannot be considered to be true control groups. Further details on the two case studies are described in the section "Assessment and comparison with the classification criteria, developmental toxicity". In addition, TCI provided further HCD covering a time span from 2001 to 2010. These data can be divided into studies conducted between 2001 and August 2006, for which the animals were obtained from animal provider 1, and in studies conducted between August 2006 and 2010, for which the animals were obtained from animal provider 2. The two case studies mentioned above as well as the study by Anonymous (2011b) obtained their animals from the same provider. RAC noted that there was an increase in incidences of PTA also in control animals in the years 2007 / 2008, with 0.19% of fetuses affected in 2007 and 0.13% of fetuses affected in 2008. As the observed incidence in the top dose group of Anonymous (2011b) still exceeded these values (incidence was 1.7%) RAC considered the finding still relevant.

Concerning the incidences of severely malaligned sternebrae, TCI referred to a statement of the conducting laboratory from 2019, referring to an evaluation from 2018, in which it was concluded that all instances of malaligned sternebrae should be classified as developmental variations, as they were considered not to alter general body conformity, not to disrupt or interfere with normal bodily function and that they are not likely to be incompatible with life. In view of this considerable change in classification, TCI re-examined the photographs of the 3 foetuses with malaligned sternebrae from the Anonymous (2011b). They concluded that only one case represented a malformation, the other two cases were considered minimal or slight. RAC is however of the view that the provided argumentation is insufficient to support a deviation from the conclusion drawn by the original examiner, who not only looked at the photographs, but also had the specimens to evaluate.

In line with the DS, RAC is of the view that the available HCD clearly indicates that both types of effects are rare events. The incidence (% per litter) for both effects either exceeded the maximum incidence observed in the HCD (severely malaligned sternebrae) or was in the upper range (persistent truncus arteriosus). When excluding a single control litter with only two viable fetuses, with one affected by PTA from the HCDs, the incidence of PTA in the top dose of the Anonymous (2011b) study also exceeds the HCD for PTA. Overall, the vast majority of studies provided as HCD did not show any incidences of these two malformations in the control groups (Persistent truncus arteriosus: 127 studies → no incidence in 113 studies, in 14 studies single litters were affected (litter being the relevant experimental unit) and in one of these 14 studies 2 foetuses in 2 litters were affected (15 of 23501 foetuses, 14 of 2675 litters); Severely malaligned sternebrae: 127 studies → no incidence in 110 Studies, in 17 studies single litters were affected (litter being

[2] https://www.ecetoc.org/wp-content/uploads/2014/08/MON-031.pdf

the relevant experimental unit) and in two of these 17 studies 2 foetuses in 2 litters were affected (19 of 23501 foetuses, 17 of 2675 litters)).

TCI also pointed out that these malformations were not seen in the other studies with fenpropidin. However, in line with the DS, RAC is of the view that a direct comparison with the other studies is not possible due to the following reasons:

- The second rabbit study was severely compromised (many maternal deaths which were not substance related, no information on purity, exposure duration was shorter than currently recommended– though heart development is considered to be complete by ~GD18, but development of sternebrae continues until after birth).
- For the two rat studies it is relevant that it is a different species and it is possible that an effect is only seen in one species but not in another. It is further noted that a dose dependent increase in incidence of incised neural arches (and at the top dose further effects on neural arches) was seen in one of the two studies – indicating that the skeleton was a target for developmental toxicity in another study and species (but among other deficiencies in this study there was no information on the purity of the test material and no firm conclusion can be drawn on the basis of this study).

Assessment and comparison with the classification criteria

Sexual function and fertility

***Table**: Relevant information on the available two-generation studies (table taken from the CLH report, slightly adapted and complemented with information from the original study reports)*

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Reproduction study (two generations/ one litter) OPPTS 870.3800 (1998), OECD 416 (2001) GLP Oral (continuous in diet) Rat, HanIbm:WIST 30/sex/group	Fenpropidin (purity 97%) 0, 25, 100, 500 and 1000 ppm (corresponding to 0, 2-3, 8-10, 42-58 and 80-126 mg/kg bw/day for males and 0, 2-5, 8-18, 45-96 and 88-205 mg/kg bw/day for females, respectively) Vehicle: laboratory animal diet Values are statistically significant, if not indicated differently. Details on statistical analysis are presented in the CLH-report.	<u>NOAEL (parental):</u> 100 ppm (11.4 mg/kg bw) ***Parental toxicity*** **<u>1000 ppm (80 mg/kg bw/day)</u>** **F0:** There were no deaths or treatment related clinical signs. ↓ body weight gain (cumulative, days 1-68): m 22%(unclear whether statistically significant), f 24%; ↓ body weight: m 12%, f 9 % (day 68) ↓ body weight gain f, gestation (cumulative, days 0-21): 16%; ↓ body weight f, gestation : 8% (day 0), 11% (day 21) ↓ body weight loss f, lactation (-12.5g; control +18.8g, days 0-21); ↓ body weight f, lactation: 12% (day 0), 22% (day 21) Exsanguinated final body weight: m: -13%, f: -9%	Anonymous 2003

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Food consumption: See summary in the text Organ weights: See summary in the text Histopathology: ↓ liver lymphohistiocytic infiltration m 8/30 (control 19/30); ↓ spleen extramedullary haematopoiesis m 8/30 (control 20/30), f 2/30 (control 21/30); ↑ adrenal cortical fatty change f 26/30 (control 6/30), not observed in males ↓ prostate lymphohistiocytic infiltration m 6/30 (control 12/30). **F1:** There were no deaths or treatment related clinical signs. ↓ body weight gain (days 1-68): m 24%, f 5% (in f not statistically significant); ↓ body weight: m 47%, f 33 % (day 1), m 30%, f 17% (day 68) ↓ body weight gain f, gestation: 19%; ↓ body weight f, gestation: 18% (day 0), 18% (day 21) ↓ body weight gain f, lactation: 93%; ↓ body weight f, lactation: 18% (day 0) and 21% (day 21) Exsanguinated final body weight: m: -25%, f: -10% Food consumption: See summary in the text Organ weights: See summary in the text Histopathology: ↓ liver lymphohistiocytic infiltration: m 11/30 (control 21/30), f 15/30 (control 22/30); ↓ spleen extramedullary haematopoiesis: m 2/30 (control 16/30), f 3/30 (control 28/30); ↑ adrenal cortical fatty change: f 21/30 (control 10/30), not observed in males ↓ prostate lymphohistiocytic infiltration: m 4/30 (control 17/30).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		<u>500 ppm (42 mg/kg bw/day)</u> **F0:** There were no deaths or treatment related clinical signs. ↓ body weight gain (days 1-68): m 18%, f 17%; ↓ body weight (day 68): m 10%, f 6%; ↓ body weight gain f, gestation: 7% (not statistically significant); ↓ body weight f, gestation: 6% (day 0), 7% (day 21) ↓ body weight gain f, lactation: 15% (not statistically significant); ↓ body weight f, lactation: 6% (day 0), 6% (day 21) Exsanguinated final body weight: m: -10%, f: -5% Food consumption: See summary in the text Organ weights: See summary in the text Histopathology: ↓ liver lymphocytic infiltration: m 9/30 (control 13/30); ↓ spleen extramedullary haematopoiesis: f 11/30 (control 21/30); ↑ adrenal cortical fatty change: f 14/30 (control 6/30), not observed in males ↓ prostate lymphohistiocytic infiltration: m 2/30 (control 12/30) **F1:** There were no deaths or treatment related clinical signs. ↓ body weight gain (days 1-68): m 9%; ↓ body weight: m 21%, f 9% (day 1), m 12%, f 5% (day 68) Exsanguinated final body weight: m: -17%, f: -5% (not statistically significant) Food consumption: See summary in the text Organ weights: See summary in the text Histopathology: ↓ spleen extramedullary haematopoiesis m 9/30 (control 16/30), f 13/30 (control 28/30); ↑ adrenal cortical fatty change: f 19/30	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		(control 10/30), Not observed in males ↓ prostate lymphohistiocytic infiltration: m 6/30 (control 17/30) **<u>100 ppm (8 mg/kg bw/day):</u>** No treatment-related findings **<u>25 ppm (2 mg/kg bw/day):</u>** No treatment-related findings ***Offspring toxicity*** <u>NOAEL (offspring):</u> 100 ppm (11.4 mg/kg bw) **<u>1000 ppm (80 mg/kg bw/day)</u>** **F1:** ↓ number of implantation sites 12.3 (control 14.1, HCD range: 12.2 – 14.2, mean: 13.0); No clinical signs during lactation; ↓ body weight gain (days 0 - 21), evident from day 0: m 37%, f 36%; ↓ body weight (day 21), m 32%, f 32% ↓ sexual maturation: m: age 28 days (control 25.3 days), body weight 48 g (control 71 g); f: age 42 days (control 32.5 days), body weight 106 g (control 102 g); Organ weights: ↓ absolute liver weight: m 37%, f 34%, ↑ relative liver weight: f 21%; ↓ absolute / relative spleen weight: m 54% / 29%, f 48% / 24%); ↓ absolute / relative thymus weight: m 45% / 16%, f 37% / 8%; ↑ relative brain weight: m 42%, f 36%; ↓ absolute brain weight: m 8%, f 8% Histopathology: ↓ liver glycogen deposition: m 12/28 (control 28/29), f 4/29 (control 12/28); ↓ liver extramedullary haematopoiesis: m 9/28 (control 21/29); f 7/29 (control 18/28); ↓ grading of spleen extramedullary haematopoiesis: m 2.2 (control 2.9), f 2.3 (control 3.0); ↑ thymus atrophy: m 8/28 (control 0/29); ↑ thymus phagocytic cells: m 19/28 (control 5/29);	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		F2: ↓ number of implantation sites: 10.3 (control 12.4, HCD range: 12.2 – 14.2, mean: 13.0); ↓ mean number of pups delivered: 9.6 (control 11.7, HCD range: 11.3 – 13.3, mean: 12.3); no effect on live birth index ; No clinical signs during lactation; ↓ body weight gain (days 0-21): m 36%, f 36%; ↓ body weight (day 21): m 32%, f 32%; Organ weights: ↓ absolute / relative liver weight: m 40% / 7.5%, f 34% / 4% (relative liver weight not statistically significant in f); ↓ absolute / relative spleen weight: m 49% / 24%, f 49% / 27%); ↓ absolute thymus weight: m 35%, f 32%; ↑ relative brain weight: m 40%, f 38%; ↓ absolute brain weight: m 7.5%, f 6% Histopathology: ↓ liver glycogen deposition: m 5/30 (control 18/27), f 2/30 (control 15/27); ↓ liver extramedullary haematopoiesis: m 5/30 (control 24/27), f 13/30 (control 25/27); ↓ grading of spleen extramedullary haematopoiesis: m 1.9 (control 2.8), f 2.2 (control 3.1); ↑ thymus phagocytic cells: m and f 18/30 (control 6/27) both sexes; **<u>500 ppm (42 mg/kg bw/day)</u>** **F1:** ↓ number of implantation sites: 12.3 (control 14.1; HCD range: 12.2 – 14.2, mean: 13.0); No clinical signs during lactation; ↓ body weight gain (days 0-21), evident from day 4: m 16%, f 17%; ↓ body weight (day 21): m 14%, f 14%; ↓ sexual maturation: m: age 26.1 days (not statistically significant); control: 25.3 days, body weight 59.8 g (control 71.3 g) f: age 37.1 days (control 32.5 days), body weight 111 g (control 103 g); Organ weights:	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		↓ absolute liver weight: m 20%, f 16%); ↓ liver glycogen deposition: m 13/24 (control 28/29), f 4/25 (control 12/28); ↓ absolute / relative spleen weight: m 25% / 11%, f 21% / 8% (not statistically significant for relative spleen weight in f); ↓ absolute thymus weight: m 19%, f 18%; ↑ thymus phagocytic cells: m 10/24 (control 5/29); ↑ relative brain weight: m 15%, f 15%; ↓ absolute brain weight: males 3%. Histopathology: ↓ liver extramedullary haematopoiesis: f 11/25 (control 18/28); ↓ grading of spleen extramedullary haematopoiesis: m 2.5 (control 2.9) **F2:** ↓ number of implantation sites: 11.7, not statistically significant (control 14.1; HCD range: 12.2 – 14.2, mean: 13.0); No clinical signs during lactation; ↓ body weight gain: Days 0-21, evident from day 4: m 12%, f 14%; ↓ body weight: day 21: m 10%, f 12% Organ weights: ↓ absolute liver weight: m 16%, f 13%); ↓ absolute / relative spleen weight: males 17% / 5%; females 20% / 8% (not statistically significant for absolute and relative weight in m, and relative weight in f); ↓ absolute thymus weight: m 14%; ↑ relative brain weight: m 12%, f 14% Histopathology: ↓ liver glycogen deposition: m 8/29 (control 18/27), f 4/29 (control 15/27); ↓ grading of spleen extramedullary haematopoiesis: m 2.3 (control 2.8);	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		100 ppm (8 mg/kg bw/day): **F1**: ↓ number of implantation sites 12.9 (not significant, control 14.1; HCD range: 12.2 – 14.2, mean: 13.0) F2: Number of implantation sites was increased compared to controls (12.8 vs 12.4 in controls). **25 ppm (2 mg/kg bw/day):** **F1** ↓ number of implantation sites 12.4 (control 14.1; HCD range: 12.2 – 14.2, mean: 13.0) F2: Number of implantation sites was equal to controls.	
Reproduction study (two generations/ one litter) OECD 416 (1983) notable deviation is lack of systemic toxicity at the highest dose GLP Oral (continuous in diet) Rat, CD (Sprague Dawley origin) 30/sex/group	Fenpropidin (purity 91%) 0, 6.25, 25, 100 ppm (corresponding to 0.4, 1.61, 6.43 and 0.50, 2.03, 8.02 mg/kg bw/day for F0 and F1 males, respectively and to 0.48, 1.91, 7.79 and 0.56, 2.35, 9.31 mg/kg bw/day for F0 and F1 females, respectively. These values represent premating period only). Vehicle: laboratory animal diet Oral (continuous in diet)	***Parental toxicity*** No effects at any dose level ***Reproductive toxicity*** No effects at any dose level ***Offspring toxicity*** No effects at any dose level	Anonymous 1987

In line with the DS, RAC considers the dietary two generation study by Anonymous (1987) as not relevant for the assessment, as the doses used were too low and no parental toxicity, reproductive toxicity or offspring toxicity was observed. At comparable doses also no toxicity was observed in the second dietary two-generation study by Anonymous (2003), which is considered reliable with only minor deviations from OECD TG 416 (see "Summary of the DS's proposal").

RAC concurs with the DS that in the study by Anonymous (2003), the parental animals of the two higher dose groups were affected by general toxicity as evidenced by statistically significant decrease in body weight and body weight gain, although no deaths or clinical signs were observed. Body weight and body weight gain was decreased in the majority of F0 and F1 males and females at 500 & 1000 ppm from day 1 – 68 (see table 2.6.6.1.1-1 from the CLH report), and was most prominent during the lactation period in females at the top dose, where a body weight loss of – 12.5 g was noted in F0 females, while an increase of 18.8g was seen in F0 control

dams. Also in top dose F1 females the body weight gain was reduced to only 7% of the control animals during lactation. It is noted that body weight of the F1 animals was considerably lower from premating stage. At 500ppm less severe effects on body weight and body weight gain were seen in parental F0 and F1 animals of both sexes from day 1 to 68 (For F1 females both body weight and body weight gain were reduced by less than 10%, for F0 females body weight change was below 10%, but reduction in body weight gain partly exceeded 10%. For F1 males body weight gain and body weight were reduced by around 10% at day 68, while for the F0 animals body weight was reduced by 10% and body weight gain by 18%.

Absolute organ weights of the F0 generation were statistically significantly reduced for the heart and liver in the two highest dose groups in males and for liver, thymus and ovaries in females. In contrast the relative organ weights of several organs were increased in the two highest or the highest dose group (depending on the organ) in F0 males and females. There were no treatment related effects on the organ weights at the two lower doses. Also in the F1 generation organ weights of males and females of the two highest dose groups were affected; while several absolute organ weights were decreased, most relative organ weights were increased. Again, there were no effects on the organ weights at the lower doses. RAC considers these changes in organ weights seen in the two top dose groups as related to the observed effects on body weight and body weight gain, like the observed histopathological changes seen in some of the organs (see table ”Relevant information on the available two-generation studies” above).

There were slight effects on food consumption (reduction) in the parental F0 generation at 500 and 1000 ppm, reaching statistical significance only occasionally. In top dose F1 males and females, food consumption was statistically significantly reduced throughout the study, while at the next lower dose it was reduced only occasionally. No treatment-related effects on food consumption were noted in the two lower dose groups of both generations. Given the irritant properties of fenpropidin observed in several studies and supported by the evidence for skin irritant, eye irritant and respiratory irritation properties it is possible that the lower food intake at higher doses was related to these properties (affecting palatability). The lower food intake might have caused or contributed to the lower body weights observed in animals of the two higher dose groups.

Also, the body weight and body weight gain of male and female F1 pups was clearly affected during the lactation period for the 500 and 1000 ppm groups. While there was no impact on post-natal day (PND) 0, body weight and body weight gain were statistically significantly decreased up until post-natal day 21 in both male and female pups (body weight: at 1000 ppm ~33%, at 500 ppm ~15%; body weight gain: at 1000 ppm ~37%, at 500 ppm ~17%, information from the original study report). The same pattern was observed in F2 pups (body weight: at 1000 ppm ~32%, at 500 ppm ~11%; body weight gain: at 1000 ppm ~36%, at 500 ppm ~13%, information from the original study report). This might be indicative of an effect on or via lactation as no weight difference between the dose groups was evident on PND0. It is also noted that the maternal body weight of the F0 generation (as well as for the later parental F1 generation) was most strongly reduced during lactation and for the top dose even a body weight decrease was recorded, though a clear differentiation of when this effect was induced is not possible (the animals were exposed during *in utero* development as well as during the lactation period). Aspects relevant for classification for lactation are discussed under “Adverse effects on or via lactation”.

In F1 pups, sexual maturation was delayed by 2.7 days in top dose males (Balano preputial separation (BPS) in controls on day 25.3, at 1000 ppm on day 28) and by 9.5 days in top dose females (Vaginal opening (VO) in controls on day 32.5, at 1000 ppm on day 42). While in male pups the body weight was lower than in controls on the day of BPS (48.4 g vs 71.3 g in controls), the body weight in female pups on the day of VO was comparable between the top dose and

controls (105.8 g vs 102.6 g in controls). Also at 500 ppm body weight and body weight gain were significantly lower than in controls during lactation for both male and female pups, but while VO was delayed by 4.6 days, BPS was hardly delayed (0.8 days). It is further noted that body weight remained lower in the top two dose groups when the F1 animals reached adulthood (see table 2.6.6.1.1-4 of the CLH report). Overall it can be concluded that body weight development was affected in F0 and F1 animals, resulting in lower pup body weights and it is likely that this was the cause of delayed maturation (delayed puberty as indicated by delayed BPS and VO). Although the effect in females of the top dose was rather severe (9.5 days) and there was no difference in body weight when comparing the values for the day of VO in controls and top dose, it can be assumed that the body weight of the top dose animals was considerably lower on the day on which VO was seen in the control animals. In line with the DS, RAC considers the delay of puberty onset to be secondary to the lower body weight.

The number of implantation sites per litter was slightly decreased in F1 and F2 litters (F0 and F1 dams), reaching statistical significance in the top dose of the F2 litters and in all but the second lowest dose group of the F1 litters (see the Table below). There was no clear dose dependence evident, but in both generations, the values were lower in the two top dose groups. All values were within the provided HCD for the F0 dams and the significant changes (decreases compared to the concurrent control) might be caused by the relatively high value for the concurrent control (at the upper range of the provided HCD). The values were outside the HCD for both top dose groups in the F1 dams. Also the number of pups delivered was slightly reduced in the two higher dose groups of both generations (see the table below). For the F2 litters it was slightly below the provided HCD range and it reached statistical significance in the top dose group.

***Table** : Number of implantation sites and pups delivered (Anonymous 2003)*

	Control	25ppm	100ppm	500ppm	1000ppm	HCD[§] range	HCD[§] mean
Implantation sites							
F0 dams	14.1	12.4*	12.9	12.3*	12.3*	12.2-14.2	13.0
F1 dams	12.4	12.4	12.8	11.7	10.3**		
Pubs delivered							
F0 dams	13.1	13.3	13.2	12.0	11.9	11.3-13.3	12.3
F1 dams	11.7	11.6	12.1	11.1	9.6##		

* p<0.05, **<0.01 (Dunnett test); p<0.01 (Chi-Square + Fisher test)

§ … 5 studies conducted between 1998 – 2000; only 1 covered data for F1 dams (145 dams, 138 litters)

There was no effect on viability of F2 pups, while a slight but statistically significant increase of dead F1 pups was seen in the top dose between days 1 and 4 post-partum, prior to culling (see the CLH report, table 2.6.6.1.1-3). In that group, one dam lost 8 pups, which might have had an impact on reaching statistical significance when compared to the control group according to the study authors. No HCD for death of pups between day 1 to 4 were located. There was no clear dose response relationship and no concordance between the F1 and F2 generations. Nevertheless, it might be that no increase in post natal deaths was seen in the F2 generation, because several fetuses were lost as indicated by the lowered number of implantation sites and number of pups delivered in these animals. Overall, these effects were considered slight and they occurred in the presence of maternal toxicity.

Absolute and relative weights of the liver, thymus and spleen of male and female F1 and F2 pups of the two top dose groups were reduced, which, according to the study authors, is likely to be related to the lower body weights. Also, the effects on brain weight (lower absolute weight, higher relative weight) appear to be caused by the lower body weights, since although the absolute weights were lower, relative brain weights increased which might be explained by the survival strategy of the mammalian organism, which protects the brain as the most essential organ for

survival. Also the observed histopathological changes, i.e. lower incidences or severity in extramedullary haematopoiesis in the spleen and the liver and liver glycogen deposition as well as an increase in thymus atrophy and presence of phagocytic cells in the thymus are likely to be related to the lower body weights, as also concluded by Anonymous (2003). The described effects on organ weights and histopathological changes were seen in the two top dose groups of male and female F1 and F2 pups (except for relative liver weight, which increased in high dose group F1 females pups, thymus atrophy, only significantly increased in males F1 pups from HD group, and thymus phagocytic infiltration, which was not seen/increased in F1 female pups) and largely correlated with reductions in body weight.

When F1 animals reached adulthood, several absolute organ weights remained significantly lower in the mid and top dose groups compared to the control group. Unlike in pups, the relative weight of some organs of males and females of the mid and top dose groups were significantly increased compared to the controls (see text above as well as table B.6.6.1.-10a, RAR volume 3, 2021).

Histopathological changes observed in adult F1 animals included a decrease of lymphohistocytic infiltration in the liver in top dose males and females, a reduction of extramedullary haematopoiesis of the spleen in the top two doses in males and females, an increase in adrenal cortical fatty change in females of the two top dose groups and a decrease in lymphohistocytic infiltration of the prostate of males of the two top dose groups.

In line with the study authors and the DS, RAC considers these changes as secondary to the lower body weights observed in mid and top dose animals. It appears that as a consequence of the lower body weight, the general development and maturation of the animals was affected.

No relevant effects were observed on mating index and fertility index, neither in the F0 nor the F1 generation.

The table below presents the results for testis spermatid and cauda epididymides sperm counts in F0 and F1 males. In some dose groups the values were statistically significantly decreased, whereas in other groups they were increased and no clear dose dependence was obvious. The DS indicated that this result could be a consequence of the higher-than-normal values in the concurrent controls (the RAR, volume 3 (2021) refers to two studies reported by the notifier where the mean number of spermatids per g testis was 71.9 million, and the mean number of sperm per g cauda epididymides was 134.2 million). The CLH report also mentions that no other effects on sperm (sperm motility and morphology) or testis histology were described, further indicating that the observed effects on sperm numbers were not relevant. RAC concurs with this conclusion.

***Table**: Sperm counts from the two-generation study by Anonymous (2003) (Table 2.6.8.3-5 from the CLH report)*

	F0					F1				
ppm	0	25	100	500	1000	0	25	100	500	1000
Sperm counts [1 x 10^6]										
Testis (spermatids)	82.6	74.6**	76.6	77.9	66.0**	77.6	80.7	82.6*	84.1**	79.2
Cauda epididymides (sperm cells)	182.6	193.8	204.8	217.7#	158.8#	176	162.1	164.2	155.8*	160.9

* $p < 0.05$, ** $p < 0.01$ (Anova + Dunnett test); # $p < 0.05$ (Kriskal-Wallis + Dunnett test)

RAC concludes that the maternal animals showed treatment related systemic general toxicity, which might have influenced some fertility and offspring parameters, i.e. the observed decrease in pup body weight gain in the two top dose groups of both generations, the effects on organ weights and some histopathological changes in the two top dose groups (more severe in the F2 generation) as well as the observed dose dependent delay in sexual maturation in males and females in the two top dose groups. There were slight decreases in implantation sites and number

of pups delivered to F0 & F1 top dose females, which were outside the HCD for the F1 females of the two top dose groups and statistically significant in the top dose. There was a slight decrease in pup survival between day 1 and 4 of lactation in the F1 but not in the F2 generation. Overall, these effects were slight and seen in the presence of maternal toxicity.

In conclusion the observed effects were considered secondary to the observed general toxicity and therefore not demonstrating an adverse effect on sexual function and fertility.

According to the CLP regulation a classification for sexual function and fertility can also be based on adverse effects on sexual organs. In this respect, it is noted that some effects on ovary weight (and pituitary weight) as well as increased numbers of ovary and follicular cysts were seen in the carcinogenicity study by Anonymous (1989; details are presented in the section on carcinogenicity). However, as these increases were not dose dependent for ovary weight and numbers of cysts and no correlation with the pituitary weight was seen, RAC considers the results not supportive for a classification for sexual function and fertility. Effects on ovaries were also not described in the other studies.

Overall RAC concurs with the DS that based on the available information **classification for sexual function and fertility is not warranted**.

Developmental toxicity

In addition to the studies listed by DS as relevant for the assessment of fenpropidin's potential to induce developmental toxicity, i.e. Anonymous (1994e), Anonymous (1981i), Anonymous (2011b) and Anonymous (1981j), RAC also considers the two generation studies (Anonymous 2003; Anonymous 1987) as well as the developmental neurotoxicity study (Anonymous 2011a) as relevant for this hazard class.

As already outlined in the section "sexual function and fertility", the two generation study by Anonymous (1987) is not considered suitable as the doses tested were too low and no relevant toxicity was induced up to the highest dose tested (100pm: m: 8.02 mg/kg bw/day; f: 9.31 mg/kg bw/day).

The results of the two generation study by Anonymous (2003) are summarised in the table "Relevant information on the available two-generation studies" above and in the text of the section on "sexual function and fertility". Effects seen in the offspring can be summarized as a decrease in pup body weight and body weight gain in the two top dose groups, a statistically significant decrease of implantation sites and number of pups delivered to F1 females of the top dose group, a slight but statistically significant decrease in pup survival between day 1 and day 4 at the top dose of the F1 generation as well as effects on some organ weights and histopathological changes (for details see section on sexual function and fertility) as well as a delay in sexual maturation. These effects occurred in the presence of maternal toxicity (body weight decrease around 20% from gestation in high dose F1 females and of 12 to 22% in the beginning and the end of lactation period, respectively, in F0 females), were not dose dependent for decrease in implantation sites and number of pups delivered (statistically significant only for top dose F2 litters and within the provided HCD) and there was no consistency between the F1 and F2 generations (decrease in pup survival was seen in F1 but not in F2 pups). In conclusion, these observations are not considered supportive for a classification for developmental toxicity.

The results of the developmental toxicity studies are summarized in the table below.

***Table**: Overview on developmental toxicity studies, table from CLH report (slightly adapted and complemented with additional information from the original study reports).*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Developmental toxicity OECD 414 (1981) Deviations: Exposure only from GD 6 to 15. Food consumption was recorded only on day 6, 11, 16 and 21. GLP Oral (gavage) Rat, Tif: RAI f (SPF) 24 mated females/group	Fenpropidin (purity 97%): 0, 10, 60 and 90 mg/kg bw/day on gestation days 6-15 Vehicle: 0.5% CMC	**Maternal toxicity** NOAEL: > 90 mg/kg No treatment related clinical signs or mortalities. No significant differences in mean maternal absolute body weight between groups at any times of the study. **90 mg/kg bw/day**: ↓ body weight gain (max decrease 11%, at days 6-16, not significant); ↓ food consumption (10% days 11-16) **60 mg/kg bw/day**: No effects **10 mg/kg bw/day**: No effects **Developmental toxicity** NOAEL: > 90 mg/kg **90 mg/kg bw/day**: No effects **60 mg/kg bw/day**: No effects **10 mg/kg bw/day**: No effects	Anonymous 1994e
Developmental toxicity Pre OECD 414 (1981) with several significant deviations (see text below the table) GLP Oral (diet) Rat, albino (SPF) 17 females /group with live foetuses (approx. 7 litters for foetal visceral examination and 10 litters for skeletal) On day 21 of gestation rats of all dose groups were assigned to two sub-groups. **Sub-group I:** Killed on day 21 of gestation and the uteri and foetuses were examined macroscopically. 10 litters were used for skeletal examination, 7 litters for visceral examination. **Sub-group II:** about 10 dams per group were allowed to litter and rear their young up until day LD 23. On the 1st, 4th, 12th and 23rd day of lactation, the	Fenpropidin (purity not reported). Mean achieved doses were: 0, 19.5, 47.5, 87.8 mg/kg bw/day on GD 7-16. Vehicle: Nafag 850 diet	***Maternal toxicity*** NOAEL (maternal): 19.5 mg/kg bw **87.8 mg/kg bw/day**: Two dams died for unknown reasons and two animals (including one that died) had vaginal bleeding; body weight loss -19.5 g (control +38.1 g) days 7-17; ↓ body weight gain 35% (days 0-21); ↓ food consumption 22% (days 7-9), 58% (days 15-17) **47.5 mg/kg bw/day**: ↓ body weight gain 34% (days 7-17), 9% (days 0-21); ↓ food consumption 16% (days 7-9), 7% (days 15-17) **19.5 mg/kg bw/day**: No effects ***Developmental toxicity*** **Results for sub-group I:** NOAEL (developmental): 47.5 mg/kg bw **87.8 mg/kg bw/day:** Mean foetal body weight: ↓ 3% Neural arches anomaly (total cases/total fetuses examined): ↑ half present: 15/112 (control: 0/104); ↑ poorly ossified: 20/112 (control 4/104); ↑ split: 32/112 (control 2/104); ↑ incised: 276/112 (control 64/104) **47.5 mg/kg bw/day**: Mean foetal body weight: ↑ 3%	Anonymous 1981i

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
dams and the young were weighed. The young of 8 litters were killed, dissected and the humid weight of the heart, liver & kidneys was determined. All dams were killed and necropsied, and the uteri were examined for implantation sites.		Neural arches anomaly (total cases/total fetuses examined): ↑ incised: 147/105 (control 64/104) **19.5 mg/kg bw/day:** Mean foetal body weight: ↓ 6% Neural arches anomaly (total cases/total fetuses examined): ↑ incised: 100/120 (control 64/104) **Results for sub-group II:** There was no effect on pup body weight, survival or organ weights observed up until day 23.	
Developmental toxicity OECD 414 (2001) GLP Oral (gavage) Rabbit: New Zealand White, Hra:(NZW) SPF 25 mated females/group	Fenpropidin (purity 96.9%) 0, 5, 10 and 20 mg/kg bw/day on GD 7-28 Vehicle: 0.5% CMC	***Maternal toxicity*** NOAEL (maternal): 10 mg/kg bw **20 mg/kg bw/day:** One female found dead (between GD 23 and 29), cause undetermined. One female aborted 8 dead fetuses and one cannibalized (GD 24) and another female delivered on GD 29. ↓ defaecation days 16-29, significantly ↓ body weight gain 64% (cumulative, significance generally archived through gestation days 7-29); non-significantly ↓ food consumption 11% (days 7-29), significant between days 19 and 29 **10 mg/kg bw/day:** Two females found dead (between GD 23 and 29), cause undetermined. No effects was considered to be test substance-related **5 mg/kg bw/day**: No effects ***Developmental toxicity*** NOAEL (developmental): 10 mg/kg bw **20 mg/kg bw/day:** ↑ incidence of persistent truncus arteriosus: 3/204 foetuses, 3/23 litters ➔ the study authors derive a value for "% per litter" of 1.7%, not significant, within historical control range: 0 – 2.1%); ↑ incidence of severely malaligned sternebrae: 3/204 foetuses, 3/23 litters ➔ the study authors derive a value for "% per litter" of 1.6%, not significant, outside of historical control range: 0 – 1%). **10 mg/kg bw/day:** No effects were considered to be test substance-related **5 mg/kg bw/day:** No effects was considered to be test substance-related	Anonymous 2011b

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Developmental toxicity
Pre OECD 414
Deviations: Exposure GD 7-19 of gestation which is shorter than currently recommended. Mortalities were high in the treated groups (15-25%) resulting in 15 to 19 pregnant females per group. Gravid uterine weight was not determined, rationale for dose selection was not given, and details on test formulation (purity was not stated), food and water quality were not reported. Food consumption was not measured.
Pre-GLP but with QA
Oral (gavage)
Rabbit: Swiss hare
20 mated females/group</td><td>Fenpropidin (purity not reported)
0, 5, 12 and 30 mg/kg bw/day on gestation days 7-19
Vehicle: 4% gum arabic</td><td>Maternal toxicity
<u>NOAEL (maternal):</u> 12mg/kg bw
13 deaths occurred in the dams during the study in all dose groups (5 mg/kg – 5dams, 12 mg/kg – 3 dams, 30 mg/kg – 4 dams) without evidence of treatment related cause.
<u>30 mg/kg bw/day</u>: body weight loss 15g (control 129.3 g) days 7-20; ↓ body weight gain 24% (days 1-30)
<u>12 mg/kg bw/day</u>: No effects
<u>5 mg/kg bw/day:</u> No effects

Developmental toxicity
<u>NOAEL (developmental):</u> 30 mg/kg bw
<u>30 mg/kg bw/day</u>: ↓ foetal body weight (7%) – possibly a consequence of maternal toxicity or a consequence of larger litter size 7.2 (control 5.7). Latter indicated by inverse relationship
<table>
<tr><th>Dose (mg/kg bw/day)</th><th>0</th><th>5</th><th>12</th><th>30</th></tr>
<tr><td>Mean no. live foetuses</td><td>5.7</td><td>6.9</td><td>7.2</td><td>7.2</td></tr>
<tr><td>Mean foetal body weight (g)</td><td>40.1</td><td>39.4</td><td>38.2</td><td>37.1</td></tr>
</table>
<u>12 mg/kg bw/day</u>: No effects
<u>5 mg/kg bw/day:</u> No effects</td><td>Anonymous 1981j</td></tr>
<tr><td>Developmental neurotoxicity study
OECD TG 426
GLP
Oral (diet)
30 Crl:WI (Han) female rats/group</td><td>Fenpropidin (96.9%)
0, 40, 100, 400 ppm (equal to 0, 3, 7, 27 mg/kg bw)

Duration of exposure: from gestation day 6 to lactation day 21</td><td><u>NOAEL (maternal neurotoxicity):</u> ≥400 ppm (27 mg/kg bw)
<u>NOAEL (developmental neurotoxicity):</u> ≥ 400 ppm (27 mg/kg bw)

<u>Maternal toxicity</u>:
No effect on survival, bw (gain) during gestation, food consumption, gestation length, number of implantations; no findings at necropsy;

Small decrease of body weight gain during lactation (LD 1-21):
27 mg/kg bw/day: -17.8% compared to control (body weight on lactation day 21: -4.1% compared to control)
7 mg/kg bw/day: -20% compared to control (body weight on lactation day 21: no effect)
3 mg/kg bw/day: no effects</td><td>Anonymous 2011a</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Offspring: No effect on the mean number of pups born, litter size, postnatal survival; no findings at necropsy No effects on nervous system: no changes in brain weights, dimensions; no histopathological alterations in central or peripheral nervous system tissues. **27 mg/kg bw/day**: mean pup body weight slightly ↓ at PND 21: 7.9% (m) and 6.2% (f), mean post-culling body weight gain (PND 4-21) slightly ↓: 8.8% (m), 7.5% (f) **7 & 3 mg/kg bw/day:** no effects Post-weaning body weights and body weight gains were unaffected in all dose groups. ***Neurotoxicity & developmental neurotoxicity:*** Absence of any effects up to 27 mg/kg bw/day	

Developmental toxicity studies in rat

In Anonymous (1994e) Tif:RAI f (SPF) rats (24 mated dams/dose) were exposed to fenpropidin (purity: 97%) at doses corresponding to 0, 10, 60 & 90 mg/kg bw/day, by oral gavage (vehicle: aqueous solution of 0.5% (CMC) carboxymethylcellulose), on GD 6-15. Dams were killed on GD 21 and fetuses were removed by caesarian section and examined. The study was conducted in accordance with OECD TG 414 (1981), with the deviation that exposure was only from GD 6-15.

Only slight maternal toxicity was seen in the top dose (non-statistically significant decrease in body weight gain between GD 6 – 16 by 11%, decrease in food consumption between GD 11 – 16 by 10%). There was no statistically significant change in number of implantation sites, post implantation losses, number of live fetuses per litter and no dead or aborted fetuses were reported. Although non-statistically significant, a dose dependent decrease of pre-implantation loss was observed. Nevertheless, as mating, ovulation and implantation occurred prior to compound administration, it can be concluded that this observation was not related to the treatment. Fetal sex ratios and body weights were not affected and there were no treatment related external, visceral or skeletal abnormalities (Anomalies were reported in both control and exposed group, but without any evident dose response relationship or statistical significance and were still within historical control values, where available). There were no findings supporting a classification for developmental toxicity.

RAC notes that testing of higher doses might have been tolerated given that only very slight effects were seen in the top dose dams, indicating that the MTD was not reached. Also, the use of a lipophilic solvent might have been preferable over an aqueous vehicle, given the lipophilicity of fenpropidin (logPow = 2.9 at 25°C, pH 7.0). Together with the shorter exposure when compared to OECD TG 414 (from GD 6-15 instead of GD 5-20), low weight should be given to the negative results of this study.

In the study by Anonymous (1981i), Albino (SPF) rat (17-18 mated dams) were exposed to dietary mean fenpropidin (purity not reported) doses of 0, 19.5, 47.5 & 87.8 mg/kg bw/day, on

GD 7-16 instead of GD 5-20. In order to ensure good mixing within the food, fenpropidin was bound to fat globules, consisting of 10% fenpropidin, 2% Ronoxan A, 0.1% 3-Carotin 20% SC and 87.9% DIMODAN PVP (grindsted).

The study pre-dated OECD TG 414 (1981) and had several deviations from the TG protocol: Only 17-18 instead of 20 pregnant rats per group were tested and only 10 and 7 litters were examined for skeletal and visceral abnormalities, respectively. The food consumption was recorded only during treatment from day 7-16 of gestation and dosing occurred only for day 7-16. Gravid uterine and cervix weight was not measured and the maternal animals were not fully necropsied. Also the stability, homogeneity and achieved concentration of test substance in the diet were not reported and the purity was not indicated.

In one set of animals (Subgroup I), dams were killed on GD 21 and uteri and fetuses were examined macroscopically. Approximately 10 litters per group were examined for skeletal abnormalities whereas, approximately 7 litters per group were examined for visceral abnormalities, which is lower than recommended according to OECD TG 414. In addition to the above-described investigations, another set of animals was observed up until lactation day (LD) 23 (Subgroup II). From this sub-group the uteri of only 10 dams per group were investigated for implantation sites and only 8 litters per group were investigated macroscopically and a small set of organ weights was determined. Further details on the study design are included in the table Overview on developmental toxicity studies above.

Maternal toxicity was seen in the mid and top dose groups as indicated by statistically significantly reduced body weight gain in the mid dose (by 34% GD 7-17, 9.6% GD 1-21) and body weight loss in the top dose between GD 7-17 (-19.5 g, statistically significant decrease compared to control). After treatment, during GD 17-21 dams of the top dose group gained more weight than the controls (by 50%), resulting in an overall decrease compared to the controls between GD 1-23 of 34.6%. No clinical signs were reported, but two dams of the top dose died (GD 19 and 21) of unknown cause (both animals had a normal number of implantations), and two animals (including one that died) had vaginal bleeding. Food consumption was markedly reduced in the top dose during treatment. A decrease during the treatment period was evident, starting with 78% of controls during the first 2 days of treatment (GD 7-9), ending at 42% of controls during the last 2 days of treatment (GD 15-17). Only a slight reduction in food consumption was seen in the mid dose group (by 7%). It is likely that problems with palatability and irritating properties of the test material at least contributed to lower food intake (see also section on "sexual function and fertility", description of the dietary two-generation study by Anonymous (2003).

There was no effect on mating success, mean number of corpora lutea, mean number of implantation sites, number of live, dead fetuses per pregnant female, sex ratio in both sub-groups and gestation period in any of the two sub-groups (note that each of these parameters was not investigated in both sub-groups). Total number of resorptions per pregnant female and percent resorptions of implantations were slightly increased in the treated animals of sub-group I (The RAR states that mainly early resorptions were seen). This effect was however not dose dependent and no information on the statistical significance was provided (see the table below). Also the numbers in the concurrent controls were rather high. Without any other effect on number of fetuses or the viability of the fetuses the finding is not considered to be adverse.

***Table**: Total number of resorptions and percent resorptions of implantations in sub-groups I & II of the study Anonymous (1981i).*

mg/kg bw/day	Control	19.5	47.5	87.8
Sub-group I				
Total # of resorptions	17	22	18	29
% resorptions of implantations	8.0	9.9	9.2	12.9
Sub-group II				
Total # of resorptions	31	24	29	35
% resorptions of implantations	14.6	11.1	13.4	16.7

The mean body weight of the pups at lactation day 1 and the weight development of the young during the lactation period were not impaired in any of the dose groups.

There were no relevant visceral findings (see the table below), but there was a dose dependent increase in incised neural arches of the cervical spine. The study authors stated that the values were within the upper limits of controls "known from previous studies" in the low dose group, but above it in mid and top dose groups. No further details on the values are included in the original study report, but the CLH report refers to HCDs provided by the notifier. However, also these HCDs could not be located by RAC.

In addition to the increase in incised neural arches, also the numbers in split, poorly ossified and/or half present were increased in the top dose groups, indicating an increase in severity (see the table below).

***Table**: Foetal examination of the rat developmental toxicity study by Anonymous (1981i)*

mg/kg bw/day	control	19.5	47.5	87.5
Skeletal examination				
Foetuses/litters	104/10	120/11	105/10	112/10
Cervical neural arches:				
half present	0	0	0	15
poorly ossified	4	1	0	20
split	2	7	2	32
incised	64	100	147	276
Foetuses/litters examined for visceral effects				
Foetuses/litters	91/7	80/7	72/7	84/7
Renal pelvis enlarged	0	2	1	2
Testicles rudimentary	0	0	1	0
Hydrocephalus internal	0	0	0	1

In contrast to the study authors, RAC considers these skeletal findings as adverse effects on development. They were seen with dose dependence and at the mid dose without severe maternal toxicity. It is further noted that, although a significant decrease of pup body weight was observed in the low and high dose groups (see table Overview on developmental toxicity studies), the changes are very low (6 and 3%, respectively), and not dose dependent, as an increase of fetal body weight is even seen at the mid dose (+3%). Therefore, it is unlikely that the changes in fetuses body weight had an impact on the dose dependent increase in incised neural arches described above.

The study authors discussed a delay in ossification as a potential cause of the observed effects on the neural arches, however RAC considers the increased occurrence of affected neural arches as isolated findings and not related to any other developmental delay, since other parts of the skeleton were not specifically affected by poor ossification after fenpropidin exposure compared to controls and pup body weight was not adversely affected.

At comparable gavage doses, no comparable effects (neither on dams nor offspring) were seen in the rat study by Anonymous (1994e). This might be explained by the different form of administration (gavage vs dietary/binding of test material to fat globules) and/or the use of different strains of rat. Another uncertainty in this regard is that the purity of the test material used by Anonymous (1981i) was not known. It is therefore unknown whether the observed developmental effects might have been caused by the test material or by an impurity. In addition, it cannot be fully excluded that some of the constituents of the "fat globules" to which the test material was adhered had an influence on the observations made.

RAC considers that the study by Anonymous (1981i) raises some concern about potential developmental toxicity, but a clear conclusion is not possible due to the uncertainties described above.

In the study by Anonymous (2011a) Crl:WI (Han) rats (30 mated dams/dose) were exposed to fenpropidin (purity: 96.9%) at dietary doses of 0, 40, 100 & 400 ppm (0, 3, 7 & 27 mg/kg bw/day), from GD 6 to lactation day 21. The study was conducted according to OECD TG 426 and is GLP compliant, with the deviation that dosing was too low.

The rationale for dose selection was provided in the full study report, which refers to a meeting between the study Sponsor and US EPA Dose Adequacy Review Team, during which the data from the rat multi-generation study (Anonymous 2003) and a preliminary developmental neurotoxicity rat study (Anonymous 2011a, Draft) were reviewed. The doses tested in this preliminary study were 0, 50, 100 & 400 ppm. At 400 ppm mean body weight gains in dams were reduced by 48% to 63% compared to controls during the first week of lactation and 14% to 19% over the 3 weeks of lactation. Mean body weight gain for the pups was reduced by 12% to 25% compared to controls during the first week of lactation and 8% to 18% over 3 weeks of lactation. No information on actual body weights was provided or to what degree this value was affected. It was anticipated that the extent of the decreased body weight gain at 400 ppm should not confound the interpretation of the developmental neurotoxicity endpoints and 40 ppm were anticipated not to cause any signs of toxicity.

It was also reported that in the preliminary study, the presence of fenpropidin and its major metabolite (CDA289267) was demonstrated (for details see the section on "lactation") and it was concluded that direct dosing of the pups was therefore not necessary. RAC calculated daily uptake per pup as 0.258 µg fenpropidin and 5.928 µg CDA289267, using 6g milk/day for 14 day old rats as recommended by Kametaka *et al*. (1974). This indicates that pups were exposed to very low levels of fenpropidin and its major metabolite (equal to 15.7 µg/kg bw/d for fenpropidin and 361.5 µg/kg bw/d for the major metabolite).

The mean maternal body weights and body weight gains during gestation were not statistically significantly different compared to the controls. The overall mean body weight gain between lactation day 1 to 21 were statistically significantly decreased for the mid and high dose groups, but without dose dependency (see table Overview on developmental toxicity studies). Mean body weight of the top dose dams on lactation day 21 was 4.1% less than the control group. Although small, this difference was calculated as statistically significant.

Mean pup body weights and body weight changes during the pre-culling period were unaffected by F0 maternal test substance exposure at all concentrations. Overall mean post-culling body weight gain (PND 4-21) was 8.8% less in males and 7.5% less in females, relative to the control group. On PND 21, the mean body weights for the 400 ppm group F1 males and females were 7.9% and 6.2% less than in the control group (statistically significant), respectively. No effects on the nervous system or behavior were observed. RAC notes that much higher doses were tolerated in the rat dietary prenatal developmental toxicity study by Anonymous (1981i) and even for much longer durations in the two-generation study by Anonymous (2003). Therefore,

RAC considers the doses applied by Anonymous (2011a) as too low, as no relevant toxic effects on parental or offspring generation were observed. RAC concludes that the MTD was not reached in this study and that less weight should be given to the negative results of this study.

Developmental toxicity studies in rabbit

In the study by Anonymous (2011b), New Zealand White, Hra:(NZW) SPF rabbits (25 mated dams/dose), were exposed to fenpropidin (purity: 96.9%) at doses corresponding to 0, 5, 10 & 20 mg/kg bw/day by oral gavage (vehicle: 0.5% CMC), on GD 7-28. According to OECD TG 414 (2001) and GLP standards, without deviations.

Dose selection was based on a preliminary study for Anonymous (2011b)) in which fenpropidin was administered at doses of 0, 10, 20, 30 & 40 mg/kg bw/day. According to the authors, doses of 30 & 40 mg/kg bw/day produced excessive toxicity in this study (excessive body weight loss, reduced food consumption, all animals of these two dose groups were terminated early). At 20 mg/kg bw/day reduced body weight gain and reduced food consumption in the latter part of the treatment period and 1 female was found dead on GD 29. Intrauterine growth, survival and development were unaffected at this dose, but no values or information on statistical analysis were provided.

In the main study, a laparo-hysterectomy was performed on GD 29 and uteri, placentae, ovaries and uterine content were examined. Maternal toxicity was evidenced in the top dose group as test substance-related statistically significantly lower mean body weight gain and/or mean body weight losses in the latter part of the treatment period (GD 12-29), which resulted in a significantly lower mean body weight gain over the overall treatment period. The effects on mean body weight gain were not of sufficient magnitude to result in statistically significantly reduced mean body weights. Decreased defecation was also noted in this dose group and was seen as early as GD 16.

One of the females from the 10 mg/kg/day group that was found dead had 11 late resorptions *in utero*, 5 of which had an open eyelid. At necropsy, this female was noted with dark red contents of the vagina and uterus and thick red contents in the cervix. The two other females (from 10 and 20 mg/kg/day group) found dead were internally normal and had 15 and 14 dead fetuses *in utero*, respectively. In the 20 mg/kg/day group, one female aborted 8 dead fetuses and 1 cannibalised fetus (in total 9 implantation sites in this female) on gestation day 24 and another delivered 5 live kits on gestation day 29 and had 1 dead fetus and 3 live fetuses *in utero*. At the scheduled necropsy on gestation day 29, no test substance-related internal findings were observed in the other dams, and no statistically significant changes on total number of corpora lutea, implantations, live fetuses, early or late resorptions, pre and post implantation loss were found in the exposed groups compared to the control group.

The study authors concluded that maternal toxicity was limited and that there was no apparent relationship between death of the exposed females and exposure to the test substance. The observed decrease in defecation might be related to the observed decrease in body weight gain. RAC concludes that the maternal toxicity seen in top dose dams was minor.

A slight decrease in mean fetal body weight was seen in the highest dose group but it was not dose dependent or statistically significant. In the top dose group developmental toxicity was observed consisting of an increase in fetuses with severely malaligned sternebra(e) and persistent truncus arteriosus (see the table below).

There was no evidence of maternal or developmental toxicity at 5 and 10 mg/kg bw/day.

***Table**: Incidences of malformation in the rabbit study by Anonymous (2011b): Numbers from the original study report.*

	Control	5 mg/kg bw/d, Foetuses (litter), % per litter	10 mg/kg bw/d, Foetuses (litter), % per litter	20 mg/kg bw/d, Foetuses (litter),% per litter	HCD**			
					mean % per litter (range)	median % per litter (range)	25th perc. % per litter	75th perc. % per litter
Fetuses (litter)	222 (23)	224 (24)	197 (20)	204 (23)	/	/	/	/
Persistent truncus arteriosus	0	0	0	3 (3), 1.7%	0.1% (0-2.1%) #	0.0%	0.0%	0.0%
Severely malaligned sternebrae	0	0	0	3 (3), 1.6%	0.1% (0-1%)	0.0%	0.0%	0.0%
Malaligned sternebrae – slight or moderate	2 (2), 0.9	4 (3), 1.7	5 (4), 2.7	3 (3) 1.3	1% (0-6.9%)	0.8%	0.5%	1.3%
Total skeletal malformation	0	1 (1), 0.5%	0	4 (4), 2%	0.9% (0-3.2%)	0.8%	0.0%	1.3%
Combined total malformation	1 (1), 0.4%	3 (3), 1.7%	3 (2), 1.5%	7 (7)* (3.6%)	2% (0-4.8%)	1.7%	1.0%	3.1%

* p<0.05, ** (69 dataset, from 17 April 2006 to 26 June 2009), # … The value of 2.08% is attributed to a single study where the affected litter had only 2 viable fetuses (50% affected). The Range without this litter is: 0 – 1.1%.

The increase for severely malaligned sternebra(e) and truncus arteriosus was not statistically significant but as these findings are considered to be rare events (no incidences in the concurrent control and in the lower dose groups, low incidence in the HCD), they are still considered relevant.

RAC agrees with the analysis provided by the DS regarding the occurrence of persistent truncus arteriosus, i.e. although just within the provided HCD the occurrence is still considered relevant as it was within in the upper range of the HCD (the mean: 0.1%, median: 0%, 75th quartile: 0%; all numbers are listed in the table above) and when one litter with only two viable fetuses, one affected by PTA, was excluded the HCD were exceeded (see the table above). RAC also agreed with the DS's assessment of the case studies on persistent *truncus arteriosus* (PTA) provided by the applicant and that these data cannot be used as HCD (see "Summary of the dossier submitter's proposal" and response to comment under "Comments received during consultation").

TCI presented 74 studies conducted from 2007 to 2012. These data not only included incidences in control studies, but also incidences in treated groups, which TCI titled "inactive/unresponsive" treatment groups as these incidences were not dose dependent. Also, the two case studies submitted earlier (Case study 1: April 2007, Case study 2: Oct 2007) were included. The observations in these 74 studies can be summarised as follows:

- Considering only the actual control groups, there were 7 of 74 studies with a single litter affected (6 studies with 1 foetus affected, 1 study with 2 foetuses affected).

- Considering the actual control groups as well as those treated groups that showed PTA without dose response [3], there were 25 studies which had 1 litter with PTA (two of these had 2 foetuses in one litter affected) and there were two studies with 3 foetuses of 3 litters affected (Case Studies 1 & 2).

As pointed out by TCI, in Chapter 9 of their textbook, Hood *et al.* (2012) describe observations of PTA in the laboratory in which the Anonymous (2011b) study had been conducted. They concluded that after the provider of the rabbits had moved, there was an increased incidence of PTA seen in the colony for about two years. During 2007 and 2008 there were 12 of 8596 control fetuses seen with PTA and overall, during this period, 37 of 34783 foetuses (in 36 litters) were seen across different dose groups. Due to this increase in occurrence of PTA subsequent to the change in provider, control data for animals obtained from the two sources was separated and slight differences were noted immediately, in terms of maternal growth parameters, fetal weights and the incidence and persistence of developmental malformations and variations. Reproductive and developmental incidences were highly conserved between the two sources, demonstrating the stability of the animal model. Fourteen male bucks which were used to sire dams in the relevant time period were removed from the colony, but the high incidence of PTA remained until the end of 2008, followed by a rather drastic decline to pre-2006 levels in the subsequent 2 year period. During 2009-2010, only two of 4312 control fetuses were noted with PTA, with an overall spontaneous incidence of 6 affected fetuses among a total of 15683 foetuses evaluated (0.05% per litter).

Hood *et al.* (2012) emphasised the need to regularly analyse historical datasets to detect secular trends and the importance of maintaining source-based historical controls.

In conclusion, RAC is of the view that the increased incidences seen in the years 2007 and 2008 should not be considered for assessing the relevance of the findings in the Anonymous (2011b) study. This is also supported by the fact that also after the Anonymous (2011b) study until 2014 the incidences remained equally low as before and after the 2007/2008 increase.

Also the two case studies (1 & 2) which were provided earlier fall within the time period where higher incidences of PTA were observed in the laboratory. More details on these studies can be found in the table below.

Table: *Relevant information from the two case studies with 3 foetuses of 3 litters affected in low and mid dose, respectively (Hood, 2012).*

Case study 1:	**Test material:**				
Apr 2007	**α-2 adrenergic agonist**	**Group 1**	**Group 2**	**Group 3**	**Group 4**
Fetuses / litters		0 / 0	3 / 3	1 / 1	0
# of fetuses		146	181	180	130
# of litters		17	20	19	17
Mean % / litter affected		0.0	1.8	0.5	0.0

[3] Studies where PTA incidences were seen only in the top dose(s) were excluded. But one study (No 44) was included as it showed a single incidence in both the control and the top dose group.

Case study 2:	Test material:				
Oct 2007	**Inert pharma-ceutical excipient**	**Group 1**	**Group 2**	**Group 3**	**Group 4**
Fetuses / litters		0 / 0	0 / 0	3 / 3	0 / 0
# of fetuses		191	184	160	194
# of litters		21	20	19	22
Mean % / litter affected		0.0	0.0	1.9	0.0

RAC rejected the use treated groups showing incidences without a dose response relationship as part of the historical control data.

RAC disagrees with the applicant's statement on the second type of developmental effect, i.e. severely malaligned sternebra(e). In this statement, the applicant considers the incidence of 3 fetuses in 3 litters in the top dose group as just outside the highest HCD incidence (2 fetuses in 2 litters, seen in two studies) and concludes that the occurrence of maligned sternebrae was more consistent with a spontaneous event rather than an effect of treatment in the absence of other skeletal effects. The DS does not present their view on this statement, but RAC is of the view that the totality of the litters from the HCD should be considered and that the increase in severely malaligned sternebra(e) in the high dose group (1.6%) compared to the highest value from the range of HCD (1%, mean 0.1%) and control (no occurrence) is of concern.

While the study authors identified severely malaligned sternebra(e) as a malformation, in contrast to slight or moderate cases, the DS referred to 'devtox.org', where malaligned sternebara(e) had been downgraded from malformation to variation. RAC notes, however, that following 'devtox.org', the effect was not considered a variation but was allocated to the grey zone between a malformation and variation. In addition, 'devtox.org' does not differentiate between minor, moderate and severe cases of malaligned sternebra(e).

RAC also noted that in the ECETOC monograph No. 031 (2002[4]), severely malaligned sternebra(e) are referred to as malformations.

In the top dose animals, there was also a statistically significant decrease in unossified sternebrae 5 and/or 6, further supporting that the sternebarae are a target of toxicity.

In addition, the study authors reported that the total number of fetuses with malformations increased with dose and that it was significantly increased compared to controls in the top dose group. It is further noted that 6 different fetuses of 6 different litters were affected. Maternal toxicity was observed at that dose, however, as described above, this toxicity was minor and not considered sufficient as cause of the described malformations. These developmental effects are therefore not considered to be secondary to maternal toxicity.

In conclusion, there was a slight increase in rare malformations just above the provided HCDs, which was not statistically significant, but the overall number of fetuses with malformations showed a dose-dependent increase, which was statistically significant at the top dose. At this

[4] https://www.ecetoc.org/wp-content/uploads/2014/08/MON-031.pdf

dose only minor maternal toxicity was observed and it can be concluded that the developmental effects are not secondary effects but are directly related to fenpropidin exposure.

In the study Anonymous (1981j), Swiss hare rabbits (20 mated dams/dose), were exposed to fenpropidin (purity not reported) at doses corresponding to 0, 5, 12 & 30 mg/kg bw/day, oral gavage (vehicle: 4% gum arabic), on GD 7-19. The study pre-dated OECD TG 414 and GLP (1981) and had several deviations: the treatment period was shorter than recommended, mortalities were high (15 – 25% in treated groups) resulting in only 15 to 19 pregnant females per group, gravid uterine weight was not determined, no rationale for dose selection was presented, purity of test material was not indicated, food and water quality were not reported and food consumption was not measured.

There were non-treatment related deaths in all dose groups (for details see section "Summary of dossier submitter's proposal"). Maternal toxicity was seen in top dose dams as indicated by body weight loss between GD 7 and 20. It is noted that, the mean weight gain was already lower in the top dose group compared to the control before treatment had started (i.e. between GD 1 – 7). Over the whole gestation period (GD 1-30) body weight gain was reduced by 24%.

In top dose fetuses body weight was slightly and non-significantly reduced by 7%, which could be a consequence of the observed effects on maternal weight or more likely a consequence of the larger litter size in the top dose compared to control (see table Overview on developmental toxicity studies). No other effects were reported. There were no findings supporting a classification for developmental toxicity. In line with the DS, RAC is of the view that this study was severely compromised and considers it supplementary only.

Discussion:

The table below gives an overview on the information relevant to assess fenpropidin's potential to induce developmental toxicity.

***Table**: Overview of the effects observed in the studies relevant to assess developmental toxicity.*

Study – species	**Dose (Purity)**	**Duration – mode**	**Limitations**	**Maternal effect at top dose**	**Developmental effects**
Anonymous 2003 Rats	2, 8, 42, 80 mg/kg bw/day (97%)	Continuous 2 generation Diet	None	Decrease in body weight and body weight gain	None
Anonymous 1987 Rats	0.4 - 0.56, 1.61 - 2.35 and 6.43 – 9.31 mg/kg bw/day 91%	Continuous 2 generation Diet	MTD not reached	None	None
Anonymous 1994e Rats	0, 10, 60 and 90 mg/kg bw/day (97%)	GD 6-15 Gavage	MTD not reached	Slight: decrease of food consumption by 10% (GD 6-16)	None
Anonymous 1981i Rats	0, 19.5, 47.5, 87.8 mg/kg bw/day (Purity not reported)	GD 7 – 16 Dietary	Purity not reported	Body weight loss between GD 7-17	Dose dependent increase in incised cervical neural arches. Additional anomalies of cervical neural arches at the top dose.

Anonymous 2011a Rats	0, 3, 7, 27 mg/kg bw (96.9%)	GD 6 – LD 21 Dietary	MTD not reached	Decrease only in BW gain in lactation between GD 1 – 21	Decrease of pups mean body weights on PND21
Anonymous 2011b Rabbit	0, 5, 10 and 20 mg/kg bw/day (96.9%)	GD 7 – 16 Gavage	None It appears that higher doses could have been tolerated, only minor maternal toxicity in the top dose group	Decrease in BW gain during the whole exposure period	Severely malaligned sternebrae (rare) Persistent truncus arteriosus (rare)
Anonymous 1981j Rabbit	0, 5, 12 and 30 mg/kg bw/day (Purity not reported)	GD 7 – 19 Gavage	Purity not reported MTD possibly not reached (high standard deviation for maternal body weight gain)	BW loss between GD 7 and 20 (SD high)	None

All five developmental toxicity studies had different kinds of deficiencies. The most recent studies were the rabbit OECD TG 414 study by Anonymous (2011b) and the rat OECD 426 study by Anonymous (2011a), but while the dose levels used by Anonymous (2011a) were clearly too low, also the doses used by Anonymous (1991b) were not high enough to fully assess the potential of fenpropidin to induce developmental toxicity (only minor effects on maternal animals were reported). It is also noted that the OECD TG 426 protocol does not foresee a detailed skeletal and visceral assessment as required according to OECD TG 414.

The remaining three studies, one in rabbits (Anonymous 1981j) and two in rats (Anonymous 1994e & Anonymous 1981i), were of differing quality, but had in common that the exposure duration was shorter than currently recommended in all three studies and ended several days before end of gestation, while according to the most recent guideline the exposure should continue up until the day prior to caesarian section.

It can be concluded that in none of the studies were the animals adequately exposed. Nevertheless, there were still some effects detectable. Despite the relatively low doses used in the rabbit study by Anonymous (2011b) there was a slight non-significant increase in two different types of malformations in the top dose group (severely malaligned sternebrae and persistent truncus arteriosus). Though close to the upper range of the HCD values it is noted that these findings are rare and no incidences were seen in the concurrent controls (and the two lower dose groups). For the malaligned sternebrae it is further noted that when the incidences of all severities are taken together a dose dependence is evident (Table Incidences of malformation in the rabbit study by Anonymous (2011b)). The statistically significant decrease in unossified sternebrae #5 and/or #6 in the top dose further indicate that the developing sternum is a target of toxicity. When total number of fetuses with malformations were taken together the increase was dose dependent and statistically significant in the top dose group.

In the rat study by Anonymous (1981i) a dose dependent increase in incised neural arches was observed, which was just inside the HCDs in the low doses but exceeded HCD at the mid and top

doses (Limited information on HCDs was provided). At the top dose further effects on neural arches were observed in addition to incised neural arches, i.e. poorly ossified, split and half present neural arches. The shorter exposure duration than that currently recommended and the small number of animals used per group would not diminish the relevance of the effects observed in the study. However, the study is considered supplementary only, because the purity of the test material was not known and for the preparation of the feed admix some unusual ingredients were used, which might have adverse effects on their own.

No relevant effects were seen in the single reliable two-generation study (Anonymous 2003), but no visceral or skeletal examinations are included in the test protocol.

Comparison with the classification criteria

There are no epidemiological data available that could support classification of fenpropidin in Category 1A.

Classification in Category 1B (presumed human reproductive toxicant) should be largely based on data from animal studies that provide clear evidence of an adverse effect on development in the absence of other toxic effects. Although the doses tested were not very high, a slight non-significant increase in two different and rare types of malformations, severely malaligned sternebrae and persistent truncus arteriosus, were seen in the reliable rabbit study by Anonymous (2011b). When total number of fetuses with malformations were taken together, the increase was dose dependent and statistically significant in the top dose group. These effects occurred in the absence of severe maternal toxicity. The other developmental effect was a dose dependent increase in incised neural arches with was statistically significant at the mid and top doses, but the study had several deficiencies, the most prominent being that the purity of the test material was not stated. Also, in this study the effects were not considered secondary to maternal toxicity.

The remaining studies had different types of deficiencies, mainly that the exposure was not adequate (mostly too low and shorter than recommended according to the current OECD 414 test guideline), and no relevant findings were reported.

RAC concludes that the observed developmental effects do not provide clear evidence of an adverse effect on development. The dose dependent increase in incised cervical neural arches in rats raises concerns, but as the study had considerable deficiencies it cannot be used for the decision on classification. However, RAC considers the slight non-statistically significant increased incidence of rare malformations, i.e. severely malaligned sternebra(e) and persistent truncus arteriosus in rabbits to be of concern. The incidences are low, but also the applied doses were rather low, and the effects are severe and rare. In conclusion RAC, in line with the DS, proposes to **classify fenpropidin as Repr. 2, H361d.**

Lactation

RAC agrees with the DS that it is likely that the observed general toxicity in the two-generation study by Anonymous (2003) in the parental animals of both generations in the two top dose groups might have caused the delayed growth in the offspring. Especially during lactation there was considerable decrease in weight gain / weight loss in dams of the two top dose groups. The DS stated that there was no indication of decreased pup viability during lactation, but RAC notes that a statistically significant increase in dead F1 pups was noted between day 1 and 4 prior to culling, but as this effect was not present in the F2 pups, RAC does not give much weight to this finding. In addition, there were effects on organ weights, histopathological changes, delays in sexual maturation in F1 and F2 male and female pups of the two top dose groups and it is likely

that these effects were a consequence of the lower pup body weight resulting from the general toxicity seen in the parental animals at these dose groups.

In contrast to the DS, RAC therefore concludes that the delay in growth only started after birth during lactation (no weight differences on day 0, information from the original study report). Together with the fact that body weight development in the dams was most severely affected during lactation, this could indicate that the impaired pup growth was at least partly caused by an effect of fenpropidin on or via lactation. However, as the animals were exposed both during *in utero* development as well as during lactation it is not possible to conclude when this effect was induced.

In contrast to the DS RAC is therefore of the view that there were no data available that would allow to conclude that the quality of the milk was not impaired and it can also not be completely ruled out that the ability of the mothers to nurse their young was affected (despite the clear growth delay in the F1 pups and the delay in sexual maturation they were able to reproduce normally). No data on the composition of the milk or the presence of fenpropidin / its metabolites was presented in the CLH report. However, RAC notes that in the neurodevelopmental toxicity study by Anonymous (2011a), the presence of fenpropidin and its main metabolite was demonstrated in the milk (Milk samples obtained on lactation day 14 from dams that consumed diet containing 400 ppm fenpropidin indicated measureable amounts of fenpropidin (0.043 ppm) and the major metabolite of fenpropidin, CDA289267 (0.988 ppm)).

In line with the DS, RAC considers the results from the littering phase of the rat developmental toxicity study by Anonymous (1981i) as not supportive for a classification for lactation (there were no effects on the investigated parameters: body weight development and survival during lactation, pup organ weights on PND 23). Also the study by Anonymous (1987) did not reveal any relevant findings for a classification for lactation, but as already stated, the doses applied in this study were too low.

Overall, RAC concurs with the DS that the available data **do not warrant classification of fenpropidin for lactation due to inconclusive data**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Fenpropidin is an active substance used in plant protection products as an agricultural fungicide to control powdery mildews, rusts, and *Rynchosprium secalis* in cereal crops. The substance is currently not listed in Annex VI to CLP (Regulation (EC) No 1272/2008).

The DS proposed to classify the substance as

- **Aquatic Acute 1** with **M-factor of 1000** based on *Desmodesmus subspicatus* 72 h E_rC_{50} value of 0.000688 mg/L and
- **Aquatic Chronic 1 with M-factor of 100** based on *Lemna gibba* 7 d NOE_rC value of 0.000599 mg/L and being not rapidly degradable.

Degradation

Under sterile aquatic conditions, fenpropidin was stable to hydrolysis at pH 3, 7, and 9 at 50ºC. Fenpropidin was thus considered hydrolytically stable.

The photolytic degradation of fenpropidin in water has been investigated under sterile conditions in aqueous buffer solution at pH 6. Photochemical transformation in water is not considered to be a significant route of degradation of fenpropidin.

The ready biodegradation of fenpropidin was determined in a modified Sturm test (OECD 301B) over 43 days at two test concentrations (10 and 20 mg/L) and 21.0-22.5 ºC. Fenpropidin was degraded in at least two steps, seen as two lag phases. A plateau of the biodegradation curve was not reached at day 41. The shapes of the curves were similar at both test concentrations, but degradation was slower at the higher test concentration. CO_2 evolution was found to be 40% (10 mg/L) and 15.1% (20 mg/L) after 43 days. Since > 20% biodegradability was observed, fenpropidin may be considered as "inherently biodegradable". The positive control was degraded by 87% within 141 hours. The ready biodegradability test indicated that fenpropidin was not readily biodegradable.

In an 'aerobic mineralisation in surface water' study performed according to OECD TG 309 the fate of fenpropidin was investigated in natural water at pH 8.4 over 61 days. The degradation rate was dependent on concentration and slower in the sterile system than the biotic systems. Mineralization of fenpropidin was not significant, mean $^{14}CO_2$ levels never exceeded 6.2% of applied radioactivity for all the incubation groups tested. The only major degradation product of fenpropidin was found to be CGA289267 which reached a maximum mean level of 25.5% of applied radioactivity at the higher rate treatment. CGA289267 was not detected in sterilised samples.

In four water/sediment systems fenpropidin rapidly dissipated from the water phase to the sediment in all systems. Degradation of fenpropidin in the whole water/sediment systems was relatively slow. Once deposited in the sediment, parent remained relatively stable as evidenced by the slow degradation over time and the lack of formation of significant metabolites. Fenpropidin reached a maximum of 58.2% of applied radioactivity in the sediment at day 44 before declining to 28.5% at 106 days. The only major degradant of fenpropidin was found to be CGA289267 which reached a maximum mean level of 19.4% of applied radioactivity in the water phase and 7.8% in the sediment phase. Carbon dioxide was a major product of metabolism in all systems reaching a maximum value of 45.4%.

The DT_{50}s in the range of 27.8 to 129 days for whole system for fenpropidin were determined.

The DS concluded that fenpropidin is not considered as rapidly degradable.

Bioaccumulation

There are two fish bioconcentration studies available.

In the first study (test method not stated), bluegill sunfish (*Lepomis macrochirus*, 1989) were tested in a flow-through system at a concentration of 0.19 mg/L of ^{14}C-fenpropidin for 28 days followed by a 14-days depuration period. For the whole fish, the steady-state bioconcentration factor (BCF) was determined to be 163.

In the second study performed according to OECD TG 305 the zebra fish (*Danio rerio*, 2006) were tested in a flow-through system at concentrations 42 and 140 µg/L of ^{14}C-fenpropidin for 4 days followed by a 4-day depuration period. BCF values related to whole body weight were calculated as mean BCF derived from BCF_{SS} and BCF_k. The BCF of 62 for the low concentration

(42 µg/L) and 145 for the high concentration (140 µg/L) were calculated, corresponding to a mean of 103.5. The BCF was dependent on the exposure concentration due to slow net uptake which is counteracted by fast test item depuration and metabolism. Depuration and/or metabolic degradation in fish is rapid as demonstrated by a mean clearance rate DT_{95} = 0.85 days.

The DS concluded that fenpropidin has a low potential for bioaccumulation as the BCFs for fish are below the criterion of 500 given in the CLP Regulation.

Aquatic Toxicity

For fenpropidin, reliable aquatic toxicity data are available for all trophic levels with a summary of the relevant information provided in the table below (the key endpoints used for hazard classification by DS are highlighted in bold). Aquatic toxicity studies using major metabolite of fenpropidin, CGA289267, are also available. The acute toxicity studies with CGA289267 indicated that CGA289267 is less toxic to fish, daphnids and algae than the parent compound fenpropidin (100-100000 x lower toxicity). Also, the available chronic toxicity study on algae with metabolite CGA289267 shows lower toxicity to algae than the toxicity of parent compound fenpropidin (~ 100000 x lower). The relevant information on aquatic toxicity for CGA289267 are provided in Table 9.4.1 – 1 in Volume 3 CP B.9 of RAR.

Table: *Summary of relevant information on aquatic toxicity of fenpropidin*

Method	Species	Endpoint	Toxicity value (mg/L)	Reference
Short-term toxicity				
Not stated	*Oncorhynchus mykiss*	96 h LC_{50}	2.57 mm	Anonymous (1981k)
EU method C.1 (92/69/EEC)	*Oncorhynchus mykiss*	96 h LC_{50}	2.84 mm	Anonymous (2006a)
Not stated	*Lepomis macrochirus*	96 h LC_{50}	1.93 mm	Anonymous (1981l)
Not stated	*Cyprinus carpio*	96 h LC_{50}	3.55 mm	Anonymous (1981m)
EU method C.1 (92/69/EEC)	*Danio rerio*	96 h LC_{50}	5.37 mm	Anonymous (2006b)
Not stated	*Daphnia magna*	48 h EC_{50}	0.54 mm	Hill (1981d)
OECD TG 202	*Daphnia magna*	48 h EC_{50}	6.15 mm	Noack (2007a)
OECD TG 201	*Desmodesmus subspicatus*	96 h E_rC_{50} 96 h E_bC_{50}	>0.001 n n.d.	Pupp & Wydra (2008)
OECD TG 201	*Desmodesmus subspicatus*	72 h E_rC_{50} 72 h E_yC_{50}	**0.000688** mm 0.000044 mm	Scheerbaum (2007a) Pickering & Allen (2018a)
OECD TG 221	*Lemna gibba*	Frond number: 7 d E_rC_{50} 7 d E_yC_{50} Dry weight: 7 d E_rC_{50} 7 d E_yC_{50}	 0.0789 mm 0.00367 mm 0.293 mm 0.00819 mm	Bebon & Wydra (2017)
OECD TG 239	*Myriophyllum spicatum*	Shoot length: 14 d E_rC_{50} 14 d E_yC_{50} Shoot wet weight: 14 d E_rC_{50} 14 d E_yC_{50} Shoot dry weight:	 2.0 mm 1.8 mm 0.77 mm 0.77 mm	Kirkwood (2018)

		14 d E_rC_{50} 14 d E_yC_{50}	0.91 mm 0.88 mm	
Long-term toxicity				
OECD TG 204	*Oncorhynchus mykiss*	21 d NOEC	0.32 n	Anonymous (1989a)
OECD TG 210, EPA OPPTS 850.1400	*Pimephales promelas**	32 d NOEC	0.0038 mm	Anonymous (2016)
OECD TG 202, Part II (1984)	*Daphnia magna*	21 d NOEC	0.32 n	Handley (1989b)
OECD 211	*Daphnia magna*	21 d NOEC	0.05 n	Noack (2007b)
BBA - Guideline Proposal 1995, OECD (1998)	*Chironomus riparius*	28 d NOEC	1.0 in	Grade (1999a)
OECD TG 201	*Navicula pelliculosa*	72 h NOE_rC 72 h NOE_yC	0.0008 mm 0.0008 mm	Grade (1993b) Howells (2015b)
OECD TG 221	*Lemna gibba*	7 d NOE_rC 7 d NOE_yC	**0.000599** mm <0.000599 mm	Bebon & Wydra (2017)
OECD TG 239	*Myriophyllum spicatum*	14 d NOE_rC 14 d NOE_yC	0.14 mm 0.14 mm	Kirkwood (2018)

* In the CLH report the wrong fish species is cited. *Pimephales promelas* should be instead *Oncorhynchus mykiss.*
mm – mean measured concentration;
n – nominal concentration;
in – initial nominal concentration;
n.d. – not determined

Acute toxicity

For fish, five acute toxicity tests with four different species (*Oncorhynchus mykiss, Lepomis macrochirus, Cyprinus carpio* and *Danio rerio*) are available. Bluegill sunfish was the most sensitive fish species tested in the acute studies, with a 96 h LC_{50} of 1.93 mg/L.

Regarding invertebrates, there were two *Daphnia magna* acute toxicity studies available. The lowest endpoint for invertebrates is the 48 h EC_{50} value of 0.54 mg/L.

There are two studies available for algae and two for aquatic plants. *Desmodesmus subspicatus* is the most sensitive species with a 72 h E_rC_{50} of 0.000688 mg/L.

From the available aquatic toxicity data, algae are the most acutely sensitive trophic group, therefore the acute aquatic classification proposed by the DS was based on the toxicity value for the freshwater green algae *Desmodesmus subspicatus* (72 h E_rC_{50} = 0.000688 mg/L). The DS proposed Aquatic Acute 1, H400, with acute M factor of 1000 ($0.0001 < E_rC_{50} \leq 0.001$ mg/L).

Chronic toxicity

Two chronic toxicity studies with fish *Pimephales promelas* were available. The lowest value was a 32 d NOEC of 0.0038 mg/L.

Long-term toxicity to invertebrates was assessed based on four available studies that were carried out with *Daphnia magna* and *Chironomus riparius*. *Daphnia magna* was the most sensitive species tested in the chronic studies, with a 21 d NOEC of 0.05 mg/L. The data for *Chironomus riparius* are available also as sediment concentrations (mg/kg).

There is one study available for algae and two for aquatic plants. *Lemna gibba* was the most sensitive species with a 7 d NOE_rC of 0.000599 mg/L.

The results of long-term aquatic toxicity studies indicate that aquatic plants are the most sensitive taxon, therefore the chronic aquatic classification proposed by the DS was based on the duckweed *Lemna gibba* toxicity study (7 d NOE_rC of 0.000599 mg/L). The DS proposed Aquatic Chronic 1, H410, with chronic M factor of 100 (0.0001 < NOE_rC ≤ 0.001 mg/L) as the substance is not rapidly degradable.

Comments received during consultation

A Member State (MS), a Company-Manufacturer and a National Authority provided comments.

The Company-Manufacturer provided detailed comments on different sections of the CLH report and the study summary of a new toxicity study with freshwater green algae *Desmodesmus subspicatus* (see additional key elements). In this study the 72 h E_rC_{50} of 0.000675 mg/L (mean measured) and 72 h E_rC_{10} of 0.000006 mg/L (mean measured) were determined. Based on the new data for algae, a higher chronic M-factor of 10000 is proposed by Company-Manufacturer. The new study will be submitted to EFSA within the on-going evaluation for the renewal of the approval of fenpropidin.

The view of the MS was that there are available lower NOEC values for algae than the NOEC value (NOE_rC = 0.000599 mg/L, *Lemna gibba*) used for the chronic classification of the substance by the DS. The lowest NOEC value could be from Scheerbaum (2007a) (NOEC = 0.000024 mg/L (nominal), *Desmodesmus subspicatus*). However, fenpropidin concentrations of the two lowest dilution levels were below the LOQ and the recovery rates in the concentrations above the LOQ decrease with decreasing concentrations. Therefore, NOEC/EC_{10} could not be derived from the study as it is not possible to validate the use of nominal concentrations nor estimate the actual concentrations of the concentrations below the LOQ. In the Pupp & Wydra (2008) study with *Desmodesmus subspicatus* (NOE_rC = 0.000032 mg/L), the same applies for the two lowest concentrations (<LOQ) but in this case the other concentrations were maintained correctly and are in the same range of recovery, allowing to express endpoints as nominal concentrations. Member State was of the opinion that even if not measured due to the concentration being below the LOQ, nominal concentrations may have been correctly maintained as these concentrations were prepared from serial dilutions made from higher concentrations. Therefore, the NOE_rC of 0.000032 mg/L (nominal) might be used for chronic classification. Based on this value the chronic M factor would be 1000 instead of 100.

The National Authority requested more information about long-term endpoints from the studies with the most acutely sensitive species *Desmodesmus subspicatus* as this data indicates a more stringent M factor of 1000.

The National Authority pointed out that mean measured E_rC_{10} value of 0.000047 mg/L for *Desmodesmus subspicatus* from the study by Scheerbaum (2007a) is available. The E_rC_{10} value was calculated by extrapolating the loss of the test substance from the third lowest treatment to the two lowest treatments where the measured concentrations were below the LOQ (0.0000488 mg/L). The National Authority was of the opinion that despite this non-standard calculation, this information indicates that the E_rC_{10} endpoint for *Desmodesmus subspicatus* could be in the concentration range from 0.00001 to 0.0001 mg/L.

For the same study, an E_rC_{20} of 0.000072 mg/L was calculated by Pickering and Allen (2018a) using four highest test concentrations only where measured concentrations were above the LOQ which supports concentration range from 0.00001 to 0.0001 mg/L. Based on this data aquatic chronic M factor would be 1000. As recommended by the CLP guidance (ECHA, 2017), the National Authority asked for calculation of E_rC_{10} for Scheerbaum (2007a) study using half the LOD for measured concentrations that are below this limit.

In addition, a 72 h NOE_rC of 0.000032 mg/L (nominal) is available for *Desmodesmus subspicatus* from the study by Pupp and Wydra (2008). In this study, the effects were observed above this test concentration with a clear dose-response relationship. Measured concentrations at the nominal treatment of 0.000032 mg/L were below the LOQ (0.0001 mg/L) but the LOD (0.000017 mg/L) is within the same concentration range as the nominal NOE_rC. Therefore, in the view of the National Authority the use of nominal or LOD as a worst case would lead to a NOE_rC in the concentration range from 0.00001 to 0.0001 mg/L indicating an aquatic chronic M factor of 1000. Based on CLP guidance (ECHA, 2017) giving preference for EC_{10} over NOEC for the purpose of hazard classification the National Authority asked DS to calculate the E_rC_{10} for this study.

The National Authority also pointed out that both of the above studies with *Desmodesmus subspicatus* meet the OECD TG 201 validity criteria and are therefore relevant to the hazard classification of fenpropidin.

The National Authority noted that there is a clear concentration-response relationship for the *Lemna gibba* study by Bebon and Wydra (2017) and based on the same reasons mentioned above asked DS to provide E_rC_{10} for the study, if available.

In regard to comments about algae studies, the DS indicated that the new algae study with fenpropidin on *Desmodesmus subspicatus* has been submitted and will be evaluated by RMS and considered in the classification of fenpropidin. Based on the study summary the 72 h E_rC_{10} from the study is 0.000006 mg/L.

The National Authority pointed out that in the fish bio-concentration study using *D. rerio* (2006) the BCF values of 1280 (low treatment) and 2900 (high treatment) appear to be normalised steady state BCF values, which are above the CLP criterion of 500, whereas the key steady state BCF values used in the CLH report from the same study are 62 and 145. Given the large discrepancy between these values, the National Authority asked for clarifications. In the view of the National Authority, it would be also useful to present lipid normalised and growth corrected kinetic BCF values. They highlighted that the conclusion on bioaccumulation potential will not impact the classification as the substance is not rapidly degradable. The DS agreed that the BCF_{ss} values from the study with *D. rerio* presented in the CLH dossier as the key values seem not to be lipid normalized. The DS has used the BCF_{ss} values normalised to 4.9% lipid (1280 and 2900) as the key values. The DS indicated that the BCF value from the study on *L. macrochirus* (1989) is not lipid normalized since lipid content was not measured in the study. As regards growth correction, data on growth are not available in the original study report on *D. rerio*. Moreover, *D. rerio* is considered as rather slower growing fish species at the test age, in comparison with other commonly used test species. Overall, the DS agreed to consider the BCFss values normalised to 4.9% lipid (1280 and 2900) as relevant for classification purposes.

Also during the consultation, a commenting Company-Manufacturer suggested to consider additional information not included in the CLH report (see section "Comments received during consultation"). In particular, a new algae toxicity study that was generated during the procedure for renewal of the approval of fenpropidin in accordance with Commission Regulation (EU) No 1107/2009.

<u>New OECD TG 201 – Toxicity to Desmodesmus subspicatus in an Algal Growth Inhibition Test with fenpropidin (2021)</u>

The test was carried out in accordance with OECD TG 201 and in compliance with GLP. Freshwater green algae *Desmodesmus subspicatus* was exposed to fenpropidin (purity 96.9 %) for 96 hours under static exposure conditions to the nominal concentrations of 10, 3.16, 1.0, 0.32, 0.10, 0.03 and 0.01 µg/L (each in 100 µL DMF/L), a solvent control (100 µL DMF/L) and a control. At the

start of the test, the measured concentrations were in the range 99 to 118 % of the nominal values. After 72 hours the recoveries ranged from below the limit of quantification (LOQ = 0.0024 µg/L) to 57% of nominal values. After 96 hours test duration, the recoveries ranged from below the LOQ to 41% of nominal values. The geometric mean measured concentrations were calculated for both 72-hour and 96-hour endpoints separately. All validity criteria of OECD TG 201 have been fulfilled. The results of the study are presented in the table below.

***Table**: Summary of toxicity of fenpropidin to Desmodesmus subspicatus*

Endpoint	**Geometric mean measured concentration (mg/L)**
Growth Rate	
72h E_rC_{10}	0.000006
96h E_rC_{10}	0.000043
72h E_rC_{50}	0.000675
96h E_rC_{50}	0.000299
Yield	
72h E_yC_{10}	Could not be determined
96h E_yC_{10}	Could not be determined
72h E_yC_{50}	0.000009
96h E_yC_{50}	0.000009
Biomass	
72h E_bC_{50}	0.000014
96h E_bC_{50}	0.000009

Interpretation of the lipid normalized BCF for fenpropidin derived from the fish bioconcentration study on D. rerio.

After the agreement of the final opinion, RAC received from the industry a clarification on the interpretation of the lipid normalised BCF for fenpropidin derived from the fish bioconcentration study on *D. rerio* (2006). Namely, that BCF values of 1280 and 2900 were calculated following the theoretical assumption that all the chemical was in the lipid fraction and a lipid content of 4.9%, resulting in the lipid BCF values of 1280 and 2900. This is not the same as the normalisation to 5% lipid as requested by OECD TG 305 (2012). According to OECD TG 305 (2012), the calculation of BCF_{KL} and the BCF_{SSL} can be done in the same way based on Equation A5.30 on p 61 of the guidance. The reported mean lipid content for the fish is 4.9%, ergo a lipid fraction (Ln) of 0.049. Therefore, the BCF values measured at 4.9% lipid content should be normalised to 5%, which results in very minor differences between the lipid normalised and non-lipid normalised values. Based on reported BCF_K values of 62.2 and 144.6, the calculated BCF_{KL} values are 64.5 and 147.6. Similarly, based on reported BCF_{SS} values of 63 and 145, the calculated BCF_{SSL} values are 64 and 148. In conclusion, the correct BCF_{SS} values normalised to

5% lipid are 64 and 148, which confirms a low potential for bioaccumulation and is below the CLP criterion of 500.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal to consider fenpropidin as not rapidly degradable as:

- The substance is hydrolytically stable at environmentally relevant pHs (pH 4-9).
- No significant degradation was observed in the ready biodegradability tests (OECD TG 301B), indicating that the substance is not readily biodegradable.
- In the surface water simulation test low mineralization was observed (6.2%). DT_{50s} values were in the range from 24 to 41 days (data from RAR). One major degradation product was formed, namely CGA289267.
- In the water/sediment studies limited mineralisation was observed for fenpropidin (45.4%). Degradation of fenpropidin in the whole water/sediment systems was relatively slow, with DT_{50s} in the range of 27.8 to 129 days. One major metabolite was formed (CGA289267).

Bioaccumulation

RAC has reviewed the clarification provided by the industry and is of the opinion that the lipid normalized steady state BCF values of 64 (40µg) and 148 (120 µg) should be considered for assessing the bioaccumulation potential of the fenpropidin. Based on this RAC concludes that fenpropidin has a low potential for bioaccumulation in aquatic organisms as BCF values in fish are below the cut-off value of 500 given in the CLP Regulation.

Aquatic toxicity

During the public consultation, study summary for a new toxicity study with algae was provided by the Company-Manufacturer. EC_{50} and EC_{10} values based on geometric mean measured concentrations were reported for toxicity study carried out on green algae *Desmodesmus subspicatus.* The algae study has been conducted according to OECD TG 201. All validity criteria have been fulfilled. RAC is of the opinion that the new toxicity study is valid and relevant for classification of the substance. According to current CLP Guidance (Version 5.0, July 2017), the endpoint based on growth rate reduction is preferred for algae. Therefore the 72-h E_rC_{50} of 0.000675 mg/L and 72-h E_rC_{10} of 0.000006 mg/L were selected to be used for classification by RAC. RAC notes that the EC_{50} and EC_{10} values from new algae study are lower than the key values used for classification of the substance for acute and chronic hazard by the DS. The new algae study affects the chronic classification of the fenpropidin as the value supports higher chronic M factor.

Two studies for sediment-dwelling invertebrate (*Chironomus riparius*) were reported in CLH report. The endpoints values presented in relation to sediment concentrations of fenpropidin (mg/kg) were not used for hazard classification by RAC.

<u>Acute toxicity</u>

RAC is of the opinion that adequate acute toxicity data are available for all three trophic levels. Algae is the most sensitive group with the lowest acute toxicity value being a 72 h E_rC50 of 0.000675 mg/L for *Desmodesmus subspicatus*. This is below the classification threshold value of 1 mg/L. Therefore, RAC concludes that a **classification as Aquatic Acute 1 (H400) is warranted with an M-factor of 1000** ($0.0001 < EC_{50} \leq 0.001$ mg/L).

Chronic toxicity

RAC is of the opinion that adequate chronic toxicity data are available for all three trophic levels. Algae is the most sensitive group with the lowest chronic toxicity value result being a 72 h E_rC_{10} of 0.000006 mg/L for *Desmodesmus subspicatus*. As this value is below the classification threshold value of 0.1 mg/L for a substance considered not rapidly degradable, RAC concludes that a **classification as Aquatic Chronic 1 (H410) is warranted with an M-factor of 10000** ($0.000001 < EC_{10} \leq 0.00001$ mg/L).

Additional references

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ANNEXES:

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

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