EUROPEAN CHEMICALS AGENCY

Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling at EU level of

5-fluoro-1,3-dimethyl-N-[2-(4-methylpentan-2-yl)phenyl]-1H-pyrazole-4-carboxamide; 2'-[(RS)-1,3-dimethylbutyl]-5-fluoro-1,3-dimethylpyrazole-4-carboxanilide; penflufen

EC Number: -
CAS Number: 494793-67-8

CLH-O-0000001412-86-233/F

Adopted

15 October 2018

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ECHA
EUROPEAN CHEMICALS AGENCY

15 October 2018

CLH-O-0000001412-86-233/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:	**5-fluoro-1,3-dimethyl-N-[2-(4-methylpentan-2-yl)phenyl]-1H-pyrazole-4-carboxamide; 2'-[(RS)-1,3-dimethylbutyl]-5-fluoro-1,3-dimethylpyrazole-4-carboxanilide; penflufen**
EC Number:	**-**
CAS Number:	**494793-67-8**

The proposal was submitted by **United Kingdom** and received by RAC on **5 July 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

United Kingdom 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 **17 October 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **1 December 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC:	**Michal Martínek**
Co-Rapporteur, appointed by RAC:	**Riitta Leinonen**

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 **15 October 2018** by **consensus**.

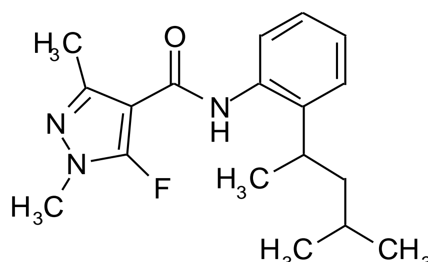
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	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	5-fluoro-1,3-dimethyl-N-[2-(4-methylpentan-2-yl)phenyl]-1H-pyrazole-4-carboxamide; 2′-[(RS)-1,3-dimethylbutyl]-5-fluoro-1,3-dimethylpyrazole-4-carboxanilide; penflufen	-	494793-67-8	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M=1 M=1	
RAC opinion	TBD	5-fluoro-1,3-dimethyl-N-[2-(4-methylpentan-2-yl)phenyl]-1H-pyrazole-4-carboxamide; 2′-[(RS)-1,3-dimethylbutyl]-5-fluoro-1,3-dimethylpyrazole-4-carboxanilide; penflufen	-	494793-67-8	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M=1 M=1	
Resulting Annex VI entry if agreed by COM	TBD	5-fluoro-1,3-dimethyl-N-[2-(4-methylpentan-2-yl)phenyl]-1H-pyrazole-4-carboxamide; 2′-[(RS)-1,3-dimethylbutyl]-5-fluoro-1,3-dimethylpyrazole-4-carboxanilide; penflufen	-	494793-67-8	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M=1 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Penflufen is a fungicidal active substance from the group of carboxamides and is intended for use in plant protection products and wood preservatives. Its structural formula is shown below.



The substance is moderately lipophilic (log K_{OW} 3.3). In orally exposed rats, penflufen is well absorbed, widely distributed, extensively metabolised (mainly via demethylation of pyrazole and hydroxylation at multiple sites) and relatively rapidly excreted.

Most of the studies with penflufen have been performed with batches of purity of about 95%. Later, in full-scale production, the purity was increased to > 98%. The impurities have been taken into consideration by the dossier submitter (DS), who did not consider them to impact on classification of penflufen.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The dossier submitter considered the data on physical hazards conclusive but not sufficient for classification based on the following information:

- Explosives: A decomposition energy of less than 500 J/g (240–330 J/g) with an onset of decomposition below 500 °C (between 270 and 410 °C) has been determined in a differential scanning calorimetry test.
- Flammable solids: In a preliminary test conducted according to A.10, the substance did not ignite on exposure to a flame but melted.
- Pyrophoric solids: Experience in handling and use indicates that penflufen is not pyrophoric.
- Substances which in contact with water emit flammable gases: Experience in handling and use indicates that penflufen does not emit flammable gases on contact with water.
- Oxidising solids: Penflufen contains oxygen and fluorine atoms but these are chemically bound only to carbon atoms. In addition, a test according to A.17 is available and considered negative.

Comments received during public consultation

No comments were received on physical hazards.

Assessment and comparison with the classification criteria

RAC concurs with the dossier submitter's assessment and their conclusion that no classification is warranted for the five endpoints evaluated in the CLH report, i.e., explosives, flammable solids,

pyrophoric solids, substances which in contact with water emit flammable gases, and oxidising solids. **No classification** for these five endpoints is **based on conclusive information**.

Two other relevant properties were not discussed by the DS: those leading to classification as self-reactive and self-heating substances. **No classification** for these two endpoints is therefore **based on lack of data**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for acute toxicity based on the following information.

Acute oral toxicity

No mortalities were observed at 2000 mg/kg bw in an acute oral toxicity study in female rats. No mortalities occurred up to 2000 mg/kg bw in either sex in an acute oral neurotoxicity study in rats.

Acute inhalation toxicity

In an acute inhalation toxicity study in male and female rats, no mortalities occurred at the highest technically achievable concentration of 2.02 mg/L.

Acute dermal toxicity

In an acute dermal toxicity study in male and female rats, no mortalities were observed at the limit dose of 2000 mg/kg bw.

Comments received during public consultation

No comments were received on this endpoint.

Assessment and comparison with the classification criteria

The available acute toxicity studies with penflufen are summarised in the table below.

Acute toxicity studies			
Type of study; **Reference (DAR);** **Year**	**Method**	**LD_{50}**	**Observations and remarks**
Acute oral toxicity, rat IIA 5.2.1/01 Year: 2007	OECD TG 423 GLP 6 females Dose: 2000 mg/kg bw	> 2000 mg/kg bw	No mortalities No clinical signs No effects on body weight No abnormalities at necropsy
Acute oral neurotoxicity, rat IIA 5.7.1/1	OECD TG 424 GLP	> 2000 mg/kg bw	No mortalities Transient clinical signs (resolved by day 3): stiff-legged gait, ataxia,

Year: 2009	12/sex/dose Doses: 0, 100, 500, 2000 mg/kg bw		decreased activity and urine staining at 500 and 2000 mg/kg bw No effects on body weight No gross pathological findings at necropsy
Acute inhalation toxicity, rat IIA 5.2.3/01 Year: 2007	OECD TG 403 GLP 5/sex/dose Concentration: 2.02 mg/L (dust aerosol) MMAD (4.1 ± 1.7) µm Nose-only, 4 hours	> 2.02 mg/L	No mortalities Generation of higher concentration and lower MMAD was not technically possible Clinical signs (persisting for up to 3 days): bradypnoea, laboured breathing, reduced motility, piloerection, red incrustations on the nose, gait high legged and staggering Lower rectal temperature after exposure (by approx. 3 °C) No adverse effects on body weight No gross pathological findings in the lung at necropsy
Acute dermal toxicity, rat IIA 5.2.2/01 Year: 2007	OECD TG 402 GLP 5/sex/dose Dose: 2000 mg/kg bw Moistened with water	> 2000 mg/kg bw	No mortalities No clinical signs of systemic toxicity No local signs of irritation No adverse effects on body weight No abnormalities at necropsy

As no mortalities occurred at the upper limit for classification of 2000 mg/kg bw in acute oral and dermal toxicity studies and no mortalities occurred at the highest technically achievable concentration in an acute inhalation toxicity study (2 mg/L), RAC agrees with the DS that **no classification** for acute toxicity is warranted.

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

Summary of the Dossier Submitter's proposal

The DS proposed no classification for STOT SE.

Although some signs of toxicity were evident following single exposure to penflufen via the oral and inhalation routes in rats, these were transient and did not lead to any significant changes in any organ. Therefore, the criteria for classification as STOT SE 1 or STOT SE 2 were not considered fulfilled. A STOT SE 3 classification was not considered justified due to the absence of conclusive signs of respiratory tract irritation or narcotic effects. The dossier submitter's analysis of the two acute studies potentially relevant for classification is summarised below.

Acute neurotoxicity study (IIA 5.7.1/1)

The reversible signs of toxicity observed in this study (stiff-legged gait, ataxia, decreased activity and urine staining at 500 and 2000 mg/kg bw) were attributed to general acute toxicity. The DS

also pointed out that a 13-week dietary neurotoxicity study in rats (IIA 5.7.1/4) showed no indications of neurotoxicity at doses up to 600 mg/kg bw/d.

Acute inhalation toxicity study (IIA 5.2.3/01)

Clinical signs observed in this study after exposure to 2.02 mg/L penflufen included bradypnoea, laboured breathing patterns, and red incrustations on the nose. According to the DS, these signs may be attributable to mechanical irritation due to inhaling a dust aerosol and do not necessarily indicate a potential for respiratory tract irritation. Gross pathological examination at necropsy did not reveal any adverse findings in the lungs that would be indicative of an irritant effect. The substance is not a skin or eye irritant. There are no repeated dose inhalation studies to investigate the respiratory irritation potential further. The remaining clinical signs seen in this study have been attributed to general toxicity.

Comments received during public consultation

One Member State Competent Authority (MSCA) was of the opinion that the STOT SE assessment would benefit from a more detailed description of the acute toxicity studies. The DS replied that the CLH report is sufficiently detailed on the clinical signs in the acute toxicity studies, and reiterated that these clinical signs were transient and did not lead to any significant functional changes in any organs.

Assessment and comparison with the classification criteria

Neurotoxicity

Incidences of clinical signs potentially indicative of neurotoxicity observed in the acute oral neurotoxicity study (IIA 5.7.1/1) are provided in the table below. Occurrence of these clinical signs was limited to days 0–3. Each dose group consisted of 12 males and 12 females.

Acute oral neurotoxicity study – incidences of selected clinical signs								
Observation	**Dose of penflufen (mg/kg bw)**							
	Males				**Females**			
	0	100	500	2000	0	100	500	2000
Ataxia	0	0	0	0	0	0	4	5
Decreased activity	0	0	0	0	0	0	4	5
Stiff-legged gait	0	0	0	0	0	0	4	5

Motor and locomotor activity was quantified as the number of beam interruptions in a figure-eight maze (see the table below). There was a dose related reduction in both sexes on day 0, more pronounced in females; this sex difference is consistent with the clinical signs. No difference between the control and treated groups was detected when the measurement was repeated on day 7.

Acute oral neurotoxicity study – group motor/locomotor activity on day 0 (after dosing)								
Parameter	**Dose of penflufen (mg/kg bw)**							
	Males				**Females**			
	0	100	500	2000	0	100	500	2000
Motor activity	569	525	329*	281*	616	376*	72*	53*
Locomotor activity	323	299	170*	153*	313	169*	28*	24*

* significantly different from control, $p \leq 0.05$

Microscopic examination of the nervous system did not reveal any treatment-related findings.

RAC notes that the abovementioned clinical signs and reductions in activity can alternatively be explained as non-specific manifestations of general toxicity.

Neurotoxicity of the compound was further investigated in a 13-week dietary neurotoxicity study (IIA 5.7.1/4) at doses up to 8000 ppm, which corresponded to 609 mg/kg bw/d in females. There were no clinical signs, no histopathologic findings in the nervous system and no effects on motor/locomotor activity.

It is also noted that no clinical signs of toxicity were reported in a 90-day rat dietary study (IIA 5.3.2/1) at doses up to ≈ 1000 mg/kg bw/d, in a mouse 90-day dietary study (IIA 5.3.2/3) at doses up to ≈ 1600 mg/kg bw/d, and in a dog 28-day dietary study (IIA 5.3.1/3) at doses up to ≈ 800 mg/kg bw/d.

Considering all the available information, RAC concludes that there is no convincing evidence of acute neurotoxicity for penflufen.

Respiratory tract irritation

No human data on respiratory tract irritation is available.Clinical signs indicative of respiratory irritation (bradypnoea, laboured breathing, red incrustations on the nose) and general toxicity (reduced motility, piloerection, staggering gait) were observed in the acute inhalation toxicity study in 4 out of 5 animals of each sex. The clinical signs persisted for up to 3 days. Necropsy did not reveal any abnormalities, but it should be noted that necropsy was performed 14 days after exposure, so transient changes such as those relating to respiratory irritation could hardly have been detected. No further inhalation studies are available.

As pointed out by the DS, the substance is not a skin or eye irritant. Only very mild reactions were observed in an *in vivo* eye irritation study. This, however, does not completely exclude the potential for respiratory irritation.

The DS further commented that the clinical signs related to the respiratory tract observed in the acute inhalation toxicity study 'are common observations during acute inhalation studies and may be attributable to mechanical irritation due to inhaling a dust aerosol, and do not necessarily indicate a potential for respiratory irritation.' Nevertheless, no data have been submitted to support this statement, so it remains speculative.

Based on the information available, RAC regards the transient bradypnoea, laboured breathing and red incrustations on the nose observed in the acute inhalation toxicity study as effects potentially relevant for classification. However, in the absence of further investigations such as histopathological or gross pathological examination shortly after exposure, the available evidence is not considered sufficiently robust to enable RAC to properly assess the nature of the effects on the respiratory tract. Therefore, classification with STOT SE 3 for respiratory tract irritation is not possible because there is limited data on this endpoint.

In summary, RAC does not find in the available dataset evidence of specific target organ toxicity following a single exposure except for clinical signs suggestive of respiratory irritation in the acute inhalation toxicity study in rats. However, the limited data on respiratory tract effects available from this study is not considered sufficiently robust to allow assessment of the nature of the observed effects. Neither were the findings observed considered to provide sufficient evidence for classification as STOT SE 1 or 2, or STOT SE 3 for narcotic effects. Therefore, RAC considers that **no classification for STOT SE** is appropriate.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on a negative *in vivo* acute dermal irritation study in rabbits.

Comments received during public consultation

No comments were received on this endpoint.

Assessment and comparison with the classification criteria

The dermal irritation study in the rabbit is summarised in the table below.

Skin irritation study		
Type of study; **Reference (DAR);** **Year**	**Method**	**Observations**
Skin irritation *in vivo*, rabbit IIA 5.2.4/01 Year: 2007	OECD TG 404 GLP 3 females 4 hour exposure Substance moistened with water	Average score for each animal (mean of 24, 48, 72 h observations): Erythema: 0, 0, 0 Oedema: 0, 0, 0

The substance did not elicit any skin reactions in this study. As the criteria for classification are not met, RAC agrees with the dossier submitter that **no classification for skin irritation/corrosion** is warranted.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on a negative *in vivo* acute eye irritation study in rabbits.

Comments received during public consultation

One MSCA supported no classification.

Assessment and comparison with the classification criteria

The eye irritation study in the rabbit is summarised in the table below.

Eye irritation study		
Type of study; Reference (DAR); Year	**Method**	**Observations**
Eye irritation *in vivo*, rabbit IIA 5.2.5/01 Year: 2007	OECD TG 405 GLP 3 females Substance pulverised	Average score for each animal (mean of 24, 48, 72 h observations): Corneal opacity: 0, 0, 0 Iritis: 0, 0, 0 Conjunctival redness: 0.7, 0.7, 0.7 Conjunctival chemosis: 0.3, 0, 0 All signs were reversible within 72 h

No effects on the cornea or iris were noted in this study. Conjunctival redness and chemosis were fully reversible within 72 hours and the average scores were below the trigger value for classification, which is ≥ 2 for both effects. As the criteria for classification were not met, RAC agrees with the DS that **no classification** for serious eye damage/irritation is warranted.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

One skin sensitisation study is available for penflufen. In this guinea pig maximisation test (GPMT), a positive response was seen in 25% of the treated animals, which is below the trigger value of 30% to consider the test positive.

One deficiency was identified by the DS in this otherwise guideline-compliant study. The topical induction dose (50%) was non-irritant, and pre-treatment with 10% sodium lauryl sulphate (SLS) in vaseline in order to create irritation was not applied in this study (although prescribed in the relevant OECD guideline).

The DS proposed no classification as the criterion of 30% response to consider the test positive was not met.

Comments received during public consultation

Two MSCAs commented on this endpoint. They considered the absence of SLS pre-treatment as a major deficiency, which together with the borderline response of 25% makes the proposal of no classification questionable. One of the MSCAs also requested a more in-depth description of the study. The DS responded that the criteria for classification do not appear to advocate making predictions of hazard based on extrapolation from deficient studies.

Assessment and comparison with the classification criteria

The guinea pig maximisation test is summarised in the following table.

<table>
<tr><th colspan="3">Skin sensitisation study</th></tr>
<tr><th>Type of study;
Reference (DAR);
Year</th><th>Method</th><th>Observations</th></tr>
<tr><td>Guinea pig maximisation test
IIA 5.2.6/01
Year: 2007</td><td>OECD TG 406
GLP
20 treated, 10 controls
Intradermal induction: 2.5% suspension in polyethylene glycol (PEG) 400
Topical induction: 50% suspension in PEG 400
Challenge: 50% suspension in PEG 400
Deficiency: The topical induction dose did not cause any irritation. In such cases the OECD 406 prescribes pre-treatment with 10% SLS. This pre-treatment was not performed.</td><td><table>
<tr><td></td><td colspan="2">No. sensitised/total no.</td></tr>
<tr><td></td><td>Control</td><td>Test</td></tr>
<tr><td colspan="3">1st challenge</td></tr>
<tr><td>48 h</td><td>0/10</td><td>5/20</td></tr>
<tr><td>72 h</td><td>0/10</td><td>4/20</td></tr>
<tr><td colspan="3">2nd challenge</td></tr>
<tr><td>48 h</td><td>0/10</td><td>2/20</td></tr>
<tr><td>72 h</td><td>0/10</td><td>0/20</td></tr>
</table>
Positive control confirmed the reliability of the test</td></tr>
</table>

RAC acknowledges that the absence of SLS pre-treatment is a major deficiency as such a pre-treatment has been shown to enhance the response to several weak sensitisers (Prinsen *et al.*, 1997), and hence had the SLS pre-treatment been conducted, the result might have been positive. Therefore, the result of the GPMT is considered equivocal by RAC.

As the only skin sensitisation study available is inconclusive due to a major methodological deficiency and no other information on the skin sensitisation potential of penflufen has been provided, RAC recommends **no classification due to inconclusive data**.

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

Summary of the Dossier Submitter's proposal

Repeated-dose toxicity of penflufen has been studied in GLP and OECD guideline-compliant studies in the rat, mouse and dog. The dossier submitter discussed findings in the liver, thyroid and pancreas.

Liver

The liver effects comprised increased weights, centrilobular hepatocellular hypertrophy and increased liver-enzyme activity and were observed in all three species tested (rat, mouse, dog). In most cases these findings occurred above the guidance values (GVs) for classification. The

liver effects at doses below the GVs were considered minimal and did not provide consistent or conclusive evidence of hepatotoxicity, and therefore did not warrant classification for STOT RE.

Thyroid

In a 28-day dog study, thyroid follicular cell hypertrophy was observed at doses below the GVs for classification. Such effects were not reported at doses relevant for classification in the 90-day or 1-year dog studies. Thyroid effects in rats were only reported at doses above the GVs for classification and there were no effects on the thyroid in the mouse studies. Since findings in the thyroid at doses relevant for classification were only seen in the 28-day dog study at low incidences and because there were only two animals per sex per dose group, this was considered by the DS to be insufficient evidence of a severe or significant adverse effect on the thyroid.

Pancreas

Exocrine single cell necrosis (minimal to slight) was observed in one 90-day study (IIA 5.3.2/1) in males at a dose relevant for classification as STOT RE 1 (9.5 mg/kg bw/d). The incidence at this dose was 5/10 vs none in controls, but was not increased at higher doses and according to the DS was within the laboratory historical control data (HCD) range. This was consistent with the incidence (3/10) seen in a follow-up 90-day study to investigate these effects (IIA 5.3.2/2), but in this study the finding was also present in the concurrent control (incidence 2/10) and in females of all groups including controls. In the 2-year rat study, no treatment-related findings were detected in the pancreas up to the top dose of 288/399 mg/kg bw/d (m/f). Further, no findings in the pancreas were reported in the mouse or dog studies. Therefore, the DS concluded that the effects seen in the rat studies were likely to be incidental and did not indicate a severe or significant toxic effect in the pancreas.

Overall, the DS concluded that penflufen does not meet the criteria for classification for STOT RE.

Comments received during public consultation

One MSCA proposed classification with STOT RE 2 (liver) due to liver injury observed in all three tested species at doses below the GVs for classification. In their interpretation, the liver injury was characterised by increased liver weight, hepatocellular hypertrophy, and clinical chemistry alterations (reduced cholesterol, increased ALP); some studies also reported hepatocellular vacuolation. The DS responded that modest changes in liver weight, increased liver hypertrophy and the induction of liver enzymes are not sufficient grounds to justify classification with STOT RE.

Assessment and comparison with the classification criteria

The repeat dose toxicity studies are summarised in the following table.

Repeat dose toxicity studies			
Type of study; **Reference (DAR);** **Year (report)**	**Method**	**Observations**	**GV for STOT RE 2**[a]
28-day dietary, rat IIA 5.3.1/1	Non-guideline Non-GLP Doses: 0, 150, 2000, 7000 ppm;	560/648 mg/kg bw/d (above GV): • ↓ bw gain (f) • ↑ liver wt (relative, by 26%/19% m/f)	300 mg/kg bw/d

Year: 2004	corresponding to 0, 12/13, 154/169, 560/648 mg/kg bw/d (m/f) 5/sex/dose Liver microsomes analysed for cytochrome P450 content and EROD, PROD and BROD activity (EROD = ethoxyresorufin *O*-deethylation; PROD = pentoxyresorufin *O*-depentylation; BROD = benzyloxyresorufin *O*-debenzylation)	• Centrilobular hepatocyte hypertrophy (in all animals, none in controls) • ↑ cholesterol (f by 31%), ↓ bilirubin (f by 45%) • ↑ cytochrome P450 (1.3/1.5-fold m/f), ↑ PROD (4.7/5.4-fold m/f), ↑ BROD (8.7/24-fold m/f); EROD unchanged 154/169 mg/kg bw/d: • ↑ liver wt (m relative by 11%) • Centrilobular hepatocyte hypertrophy (f 2/5 vs none in controls) • ↑ cholesterol (f by 27%) • ↑ cytochrome P450 (1.1-fold), ↑ PROD (2.4/1.9-fold m/f), ↑ BROD (4.3/6.5-fold m/f) 12/13 mg/kg bw/d: • No effects	
29/30-day dietary immunotoxi-city, rat IIA 5.8.2/1 Year: 2008	EPA OPPTS 870.7800 GLP Doses: 0, 200, 1000, 7000 ppm; corresponding to 0, 18/20, 83/104, 756/960 mg/kg bw/d (m/f) 8/sex/dose Parameters investigated: spleen and thymus weights, spleen cell counts, immune response to sheep erythrocytes	756/960 mg/kg bw/d (above GV): • ↓ bw gain and food cons. ≤ 83/104 mg/kg bw/d: • No effects	300 mg/kg bw/d
90-day dietary, rat IIA 5.3.2/1 Year: 2006	OECD TG 408 GLP Doses: 0, 150, 7000, 14000 ppm; corresponding to 0, 9.5/11.4, 457/492, 949/1009 mg/kg bw/d (m/f) 10/sex/dose	949/1009 mg/kg bw/d (above GV): • ↓ bw gain (f) • ↑ liver wt (relative by 64%/39% m/f) • Centrilobular hepatocyte hypertrophy (in 19/20 animals vs none in controls) • ↑ cholesterol (by 58%/27% m/f), ↓ bilirubin (f by 43%), ↑ γGT • Thyroid follicular cell hypertrophy (m 8/10, f 6/10 vs none in controls) • Pancreas exocrine single cell necrosis (m 4/10, f 4/10 vs none in controls) 457/492 mg/kg bw/d (above GV): • ↓ bw gain (f)	100 mg/kg bw/d

		• ↑ liver wt (relative by 35%/26% m/f) • Centrilobular hepatocyte hypertrophy (in all animals vs none in controls) • ↑ cholesterol (f by 36%), ↓ bilirubin (f by 35%), ↑ γGT • Thyroid follicular cell hypertrophy (m 8/10 vs none in controls) • Pancreas exocrine single cell necrosis (m 4/10 vs none in controls) 9.4/11.4 mg/kg bw/d: • Pancreas exocrine single cell necrosis (m 5/10 vs none in controls)	
90-day dietary, rat IIA 5.3.2/2 Year: 2006	Complementary study to IIA 5.3.2/1 OECD TG 408 GLP Doses: 0, 50, 150, 3500 ppm; corresponding to 0, 3.2/3.7, 9.3/11.4, 228/260 mg/kg bw/d (m/f) 10/sex/dose Deviation: only the kidney, liver, pancreas, pituitary and thyroid were examined microscopically	228/260 mg/kg bw/d (above GV): • 1 mortality (not considered to be treatment-related) • ↑ liver wt (relative by 16%) • Centrilobular hepatocellular hypertrophy (m 2/9, f 5/10 vs none in controls) ≤ 9.3/11.4 mg/kg bw/d: • No effects	100 mg/kg bw/d
90-day dietary neurotoxicity, rat IIA 5.7.1/4 Year: 2009	OECD TG 424 GLP Doses: 0, 250, 2000, 8000 ppm; corresponding to 0, 16.0/19.9, 126/156, 516/609 mg/kg bw/d (m/f) 12/sex/dose	516/609 mg/kg bw/d (above GV): • ↓ bw gain and food consumption • ↑ liver wt (relative by 23%/28% m/f) 126/156 mg/kg bw/d (above GV): • ↓ food cons. (f) • ↑ liver wt (relative by 13%/12% m/f) 16.0/19.9 mg/kg bw/d: • No effects	100 mg/kg bw/d
1-year dietary, rat (part of a carcinogenicity study) IIA 5.5.2/1 Year: 2009	OECD TG 453 GLP Doses: 0, 100, 2000, 7000 ppm; corresponding to 0, 4.6/6.3, 90/126, 327/446 mg/kg bw/d (m/f)	After 1 year dosing: 327/446 mg/kg bw/d (above GV): • ↑ liver wt (relative by 25%/30% m/f) • Centrilobular to panlobular hepatocellular hypertrophy (m 10/10, f 9/10 vs none in controls)	25 mg/kg bw/d

	Dosing for 1 year: 10/sex/dose Dosing for 1 year followed by 13 weeks recovery: 10/sex/dose Histopathology on the liver, lung, kidney, and thyroid gland of all dose groups; for all other organs only control and high dose group were examined	• Liver hepatocellular macrovacuolation, mainly centrilobular, diffuse (m 7/10 vs 1/10 in controls) • Thyroid follicular cell hypertrophy, diffuse (m 3/10, f 3/10 vs none in controls) • ↓ bilirubin (by 50%/59% m/f) 90/126 mg/kg bw/d (above GV): • ↑ liver wt (f relative by 10%) • ↓ bilirubin (f by 41%) 4.6/6.3 mg/kg bw/d: • No effects After 1 year dosing + 13 weeks recovery: 327/446 mg/kg bw/d (above GV): • ↓ bilirubin (m by 29%) • ↑ thyroid wt (m by 19%) ≤ 90/126 mg/kg bw/d (above GV): • No effects	
2-year dietary, rat IIA 5.5.2/1 Year: 2009	OECD TG 453 GLP Doses: 0, 100, 2000, 7000 ppm; corresponding to 0, 4.0/5.6, 79/113, 288/399 mg/kg bw/d (m/f) 60/sex/dose Microscopic examination carried out in all organs in all dose groups	Non-neoplastic findings (neoplastic findings are reported in the carcinogenicity section) 288/399 mg/kg bw/d (above GV): • ↓ bw gain (f by 18%) • ↓ reticulocytes • ↓ bilirubin • Hepatocellular hypertrophy, panlobular to centrilobular (m 50/60, f 47/60 vs none in controls) • Hepatocellular macrovacuolation, diffuse, mainly centrilobular (m 23/60, f 30/60 vs none in controls) • Liver focal brown pigment (m 23/60, f 30/60 vs none in controls) • Liver eosinophilic foci of cellular alteration (f 39/60 vs 27/60) • Thyroid diffuse follicular hypertrophy (m 3/60, f 3/60 vs none in controls) • Thyroid colloid alteration (m 48/60 vs 25/60, f 29/60 vs 2/60) • Ovary tubulostromal hyperplasia (f 7/60 vs 3/60 in control) 113/79 mg/kg bw/d (above GV): • ↓ bilirubin • Hepatocellular hypertrophy, panlobular to centrilobular (m	12.5 mg/kg bw/d

		21/60, f 22/60 vs none in controls) - Hepatocellular macrovacuolation, diffuse, mainly centrilobular (m 9/60, f 18/60 vs none in controls) - Liver focal brown pigment (m 9/60, f 18/60 vs none in controls) - Liver eosinophilic foci of cellular alteration (f 46/60 vs 27/60) - Thyroid colloid alteration (f 17/60 vs 2/60) 4.0/5.6 mg/kg bw/d: - Hepatocellular hypertrophy (m 5/60 vs none in controls) - Eosinophilic foci of cellular alteration (f 38/60 vs 27/60)	
28-day dietary, mouse IIA 5.3.1/2 Year: 2005	Similar to OECD TG 407 Non-GLP Doses: 0, 750, 3500, 7000 ppm; corresponding to 0, 26/31, 632/741, 1274/1585 mg/kg bw/d (m/f) 5/sex/dose	1274/1585 mg/kg bw/d (above GV): ↑ liver wt (relative by 24%/28% m/f) ↓ cholesterol (by 58%/44% m/f) ↑ ALP (f 1.3-fold) - Hepatocellular hypertrophy (m 1/5, f 3/5 vs none in controls) 632/741 mg/kg bw/d (above GV): ↑ liver wt (relative by 14%/32% m/f) ↓ cholesterol (by 52%/51% m/f) 26/31 mg/kg bw/d: - No effects	300 mg/kg bw/d
90-day dietary, mouse IIA 5.3.2/3 Year: 2006	OECD TG 408 GLP Doses: 0, 750, 3500, 7000 ppm; corresponding to 0, 26.9/31.5, 638/757, 1238/1600 mg/kg bw/d (m/f) 10/sex/dose	1238/1600 mg/kg bw/d (above GV): ↑ liver wt (relative by 23%/32% m/f) ↓ cholesterol (by 45%/60% m/f) - Hepatocellular hypertrophy (m 9/10 vs 1/10, f 7/10 vs 0/10) 638/757 mg/kg bw/d (above GV): ↑ liver wt (relative by 16%) ↓ cholesterol (by 35%/57% m/f) - Hepatocellular hypertrophy (m 4/10 vs 1/10; f 4/10 vs 0/10) 26.9/31.5 mg/kg bw/d: - No effects	100 mg/kg bw/d
18-month dietary, mouse IIA 5.5.3/1 Year: 2009 (The findings from the interim	OECD TG 451 GLP Doses: 0, 100, 1000, 6000 ppm; corresponding to 0, 14.3/18.4, 146/182, 880/1101 mg/kg bw/d (m/f)	Non-neoplastic findings (neoplastic findings are reported in the carcinogenicity section): 880/1101 mg/kg bw/d (above GV): ↑ liver wt (relative by 20%/24% m/f)	16.7 mg/kg bw/d

sacrifice after 12 months omitted here because of the lack of histopath. investigations)	50/sex/dose	• Centrilobular hepatocellular hypertrophy (m 46/48, f 31/50 vs none in controls) • Diffuse hepatocellular vacuolation (m 19/48 vs 10/48, f 44/50 vs 38/50); diffuse hepatocellular macrovacuolation, mainly periportal (m 1/48 vs 0/48; f 41/50 vs 14/50) • Thyroid follicular cell hyperplasia (f 38/50 vs 23/50 in controls) 146/182 mg/kg bw/d (above GV): • Centrilobular hepatocellular hypertrophy (m 29/49, f 5/50 vs none in controls) 14.3/18.4 mg/kg bw/d: • Centrilobular hepatocellular hypertrophy (m 13/49 vs none in controls)	
28-day dietary, Beagle dog IIA 5.3.1/3 Year: 2005	Non-guideline Non-GLP Doses: 0, 1300, 6500, 26000 ppm; corresponding to 0, 49/52, 244/246, 759/895 mg/kg bw/d (m/f) 2/sex/dose	759/895 mg/kg bw/d (above GV) and 244/246 mg/kg bw/d: • ↓ bw gain and food cons. • ↑ liver wt • ↑ ALP • Centrilobular hepatocellular hypertrophy (none in controls) • Thyroid follicular cell hypertrophy (none in controls) 49/52 mg/kg bw/d: • No effects	300 mg/kg bw/d
90-day dietary, Beagle dog IIA 5.3.2/4 Year: 2008	OECD TG 409 GLP Doses: 0, 180, 1800, 18000 ppm; corresponding to 0, 5.6/6.1, 55.7/63.1, 532/568 mg/kg bw/d (m/f) 4/sex/dose	532/568 mg/kg bw/d (above GV): • ↓ bw gain and food cons. (f) • ↑ liver wt (relative by 37%/50% m/f) • ↑ ALP (4-fold) • Diffuse panlobular hepatocellular hypertrophy (none in controls) • Hepatic perilobular multifocal single cell death (none in controls) • Adrenals diffuse cortical hypertrophy/hyperplasia (m, none in controls) 55.7/63.1 mg/kg bw/d: • Diffuse panlobular hepatocellular hypertrophy (m 1/4, f 3/4, none in controls)	100 mg/kg bw/d
1-year dietary, Beagle dog IIA 5.3.2/5	OECD TG 452 GLP Doses: 0, 200, 1000, 10000 ppm;	357/425 mg/kg bw/d (above GV): • ↓ bw gain (f) • ↑ liver wt (relative by 32%/51% m/f)	25 mg/kg bw/d

Year: 2009	corresponding to 0, 6.8/7.7, 32/38, 357/425 mg/kg bw/d (m/f) 4/sex/dose	• ↑ ALP (up to 4/7-fold m/f) • Panlobular hepatocellular hypertrophy • Intrahepatocellular brown pigment • Thyroid follicular cell hypertrophy 32/38 mg/kg bw/d: • ↑ liver wt (f relative by 28%) • Panlobular hepatocellular hypertrophy (f 1/4 vs none in controls) • Intrahepatocellular brown pigment (m 1/4 vs none in controls) 6.8/7.7 mg/kg bw/d: • No effects	
28-day dermal, rat IIA 5.3.3/1 Year: 2009	OECD TG 410 GLP Doses: 0, 100, 300, 1000 mg/kg bw/d Moistened with water 6 h/day, 5 days per week, for 4 weeks 10/sex/dose	1000 mg/kg bw/d (above GV): • Thymus increased lymphocyte debris within the thymic cortices (m 7/10, f 7/10, none in controls) ≤ 300 mg/kg bw/d: • No effects	600 mg/kg bw/d

[a] The GVs are based on a standard 90-day toxicity study. For extrapolation to different study durations, the CLP regulation recommends using dose/exposure time extrapolation similar to Haber's rule for inhalation, but this assessment should be done on a case-by-case basis (CLP, Annex I, 3.9.2.9.5). Values extrapolated using Haber's rule are provided in the last column of the table. However, Haber's rule is based on the assumption that the effective dose is inversely proportional to the duration of exposure. This assumption is obviously not valid for thyroid follicular cell hypertrophy and at most only partly valid for liver hypertrophy seen in the repeat dose studies with penflufen, so for these particular effects extrapolation using Haber's rule is not considered appropriate.

Liver

Liver is clearly a target organ of penflufen in the rat, mouse and dog. The overall picture is consistent with liver enzyme induction as evidenced by:

- Liver weight increases (rat, mouse, dog);
- Hepatocyte hypertrophy (rat, mouse, dog);
- Increased phase I and phase II liver enzyme activity (rat, mouse) shown in the mechanistic studies performed to elucidate the carcinogenic mode of action (MoA).

Further observed changes consistent with liver enzyme induction include:

- Increased ALP (dog). An increase in serum ALP is generally associated with hepatic microsomal enzyme induction in the dog and is considered adaptive if observed with associated increased liver weight and histological hepatocellular hypertrophy but without hepatocellular degeneration (Hall *et al*., 2012);
- Reduced bilirubin (rat), probably reflecting increased conjugation due to UDP Glucuronosyltransferase (UDPGT) induction.

It is of note that the liver effects in the 28-day studies did not progress to liver damage (i.e., degenerative or necrotic changes) after long term administration of comparable doses. Therefore, for the purpose of STOT RE classification, they have to be considered as truly adaptive, non-

adverse effects. The fact that long-term liver hypertrophy might be associated with increased incidence of liver tumours is addressed under the carcinogenicity endpoint.

Increases in liver hepatocellular vacuolation occurred only above the GVs for classification in the rat and mouse carcinogenicity studies. The vacuolation was of the macrovesicular type in the rat and probably also in the mouse, and the maximum severity (where known) was 'moderate'. Thus, hepatocellular vacuolation is not considered to trigger classification.

Overall, RAC considers the liver effects observed in the repeated dose studies with penflufen below the GVs for classification as non-adverse, adaptive responses not justifying a STOT RE classification.

Thyroid

Thyroid follicular cell hypertrophy was observed in several rat and dog studies and thyroid follicular cell hyperplasia was seen in the chronic mouse study. Since all affected groups showed liver hypertrophy, and UDPGT induction by penflufen has been demonstrated in the rat (see the mechanistic studies in the carcinogenicity section of the CLH report), a plausible explanation is that thyroid follicular cell hypertrophy/hyperplasia was secondary to induction of hepatic UDP-glucuronosyltransferases involved in the elimination of thyroid hormones. However, it is noted that other potential MoAs have not been investigated.

Only in one study, the 28-day dog study (IIA 5.3.1/3), thyroid follicular cell hypertrophy occurred below a GV dose for classification in Category 2 (*i.e.* at ≈ 240 mg/kg bw/d). This study used 2 animals per sex per group and follicular cell hypertrophy was observed in 1 out of 2 animals of each sex at the dose level in question compared to zero incidence in controls. No effect on the thyroid was reported at ≈ 550 mg/kg bw/d in a 90-day dog study (IIA 5.3.2/4) performed by the same laboratory. The severity of the finding at ≈ 400 mg/kg bw/d after 1-year administration (IIA 5.3.2/5) was slight to minimal while the incidence did not increase compared to the 28-day study (males 1/4 vs 0/4 in controls, females 3/4 vs 1/4 in controls). Thus, there was no progression of the lesion and no reduction in the threshold for the effect with time for up to 1 year in the dog, which not only reduces the concern but also raises questions about the applicability of Haber's rule in this case.

Haber's rule is used to extrapolate the GVs set for 90-day studies (CLP, Annex I, tables 3.9.2 and 3.9.3) to different study durations. Haber's rule is based on the assumption that the effective daily dose is inversely proportional to the duration of treatment. However, this assumption is not always valid and penflufen-induced thyroid follicular cell hypertrophy observed in the animals studies is obviously one of the effects not following Haber's rule. Therefore in this particular case, RAC gives preference to the default guidance values set for 90-day studies. As no thyroid effects occurred below 100 mg/kg bw/d in the oral animal studies and no thyroid effects occurred in the dermal study, a STOT RE classification for thyroid effects is not justified.

Pancreas

The incidences of exocrine single cell necrosis in the two rat 90-day studies are summarised in the following table.

Incidences of pancreatic exocrine single cell necrosis in the two 90-day rat studies (9-10 animals per group)						
Dose (ppm)	**0**	**50**	**150**	**3500**	**7000**	**14000**
Dose (mg/kg bw/d)	0	≈ 3.5	≈ 10	≈ 240	≈ 470	≈ 980
Males						
Study IIA 5.3.2/1	0	–	5	–	4	4

Study IIA 5.3.2/2	2	3	3	4	–	–
Females						
Study IIA 5.3.2/1	0	–	0	–	0	4
Study IIA 5.3.2/2	3	1	1	2	–	–

Taking into account:

- The lack of a dose-response relationship;
- The high background incidence as evidenced by the follow-up study IIA 5.3.2/2;
- The lack of any increase in the incidence of exocrine cell necrosis in the 2-year rat carcinogenicity study up to ≈ 300 mg/kg bw/d;
- The lack of effects on the pancreas in the two other species tested (the mouse and the dog),

RAC concludes that there is not sufficient evidence to consider pancreas as a target organ of penflufen.

In summary, RAC does not consider the findings in the liver, thyroid and pancreas to present sufficient evidence for classification for STOT RE. There were no other findings indicating a need for a STOT RE classification. Therefore, RAC agrees with the dossier submitter that **no classification for STOT RE** is warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Penflufen was negative in a standard *in vitro* genotoxicity battery consisting of an Ames test, a chromosome aberration test and a HPRT assay. Another set of *in vitro* studies was conducted later with a newer batch of the substance, reflecting a change in the impurity profile, again with negative results. In addition, the DS summarised an *in vivo* bone marrow micronucleus test with the older batch, which was also negative.

As the data do not indicate mutagenic potential *in vitro* nor *in vivo*, the dossier submitter proposed no classification for this endpoint.

Comments received during public consultation

One MSCA supported no classification for mutagenicity.

Assessment and comparison with the classification criteria

The available genotoxicity studies are summarised in the table below.

Genotoxicity studies		
Type of study; Reference (DAR); Year	**Method**	**Observations**
In vitro		
Ames test IIA 5.4.1/1 Year: 2007	OECD TG 471 GLP *S. typhimurium* TA98, TA100, TA102, TA1535, TA 1537 Plate incorporation and pre-incubation methods Tested up to the limit concentration of 5000 µg/plate	Negative ± S9
Ames test IIA 5.4.1/2 Year: 2009	OECD TG 471 GLP *S. typhimurium* TA98, TA100, TA102, TA1535, TA 1537 Plate incorporation and pre-incubation methods Tested up to the limit concentration of 5000 µg/plate	Negative ± S9
Chromosomal aberration test *in vitro* IIA 5.4.1/3 Year: 2007	OECD TG 473 GLP Chinese hamster V79 cells Short-term treatment: –S9 for 4 h, up to 70 µg/ml; +S9 for 4 h, up to 90 µg/ml Long-term treatment: –S9 for 18 h, up to 12 µg/ml	Negative ± S9 Cytotoxicity was present in all experiments, but the percent reduction was less (to 58–74%) than required (to 50%)
Chromosomal aberration test *in vitro* IIA 5.4.1/4 Year: 2009	OECD TG 473 GLP Chinese hamster V79 cells Short-term treatment: –S9 for 4 h, up to 37.5 µg/ml; +S9 for 4 h, up to 75 µg/ml Long-term treatment: –S9 for 18 h, up to 18.8 µg/ml	Negative ± S9 Cytotoxicity in the short-term treatments is considered sufficient, in the long-term treatment slightly lower than required
HPRT test IIA 5.4.1/5 Year: 2007	OECD TG 476 GLP Chinese hamster V79 cells Exposure 5 hours, up to 150 µg/ml	Negative ± S9 The concentrations were sufficiently high (cytotoxicity)
HPRT test IIA 5.4.1/6 Year: 2009	OECD TG 476 GLP Chinese hamster V79 cells Exposure 4 hours, up to 125 µg/ml	Negative ± S9 The concentrations were sufficiently high (precipitation and cytotoxicity)

In vivo		
Bone marrow micronucleus test IIA 5.4.2/1 Year: 2007	OECD TG 474 GLP Mouse, male 5/dose Two i.p. doses administered on consecutive days at 250, 500, 1000 mg/kg bw/d Animals killed 24 h after the second dose The doses were chosen on the basis of a preliminary study in which mortalities were observed at 2000 mg/kg bw/d	Negative Clinical signs of toxicity were observed at all dose levels An increase in the NCE/PCE ratio (corresponds to a decrease in the PCE/NCE ratio) indicated bone marrow exposure

As penflufen was negative in a standard set of *in vitro* genotoxicity studies and in an *in vivo* micronucleus test, RAC agrees with the dossier submitter that **no classification for mutagenicity** is warranted.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenic potential of penflufen has been investigated in a 2-year rat study and in an 18-month mouse study. Several studies have also been conducted to investigate the MoA for rat liver tumours and their relevance to humans.

Rat carcinogenicity study

In the rat, incidences of four tumour types were increased above HCD ranges, although without statistical significance in some cases:

- Hepatocellular adenoma in females
- Ovarian tubulostromal adenoma
- Brain astrocytoma in males
- Histiocytic sarcoma in males

The DS considered the increased incidence of hepatocellular adenomas in females as possibly treatment-related as it was accompanied by a concomitant increase in eosinophilic foci of cellular alteration (a preneoplastic change) and the liver is clearly a target organ of penflufen. On the other hand, no such effect was observed in males and the finding in females could alternatively be attributed to increased survival.

The incidence of ovarian tubulostromal adenomas in the top dose females marginally exceeded the HCD ranges, so these benign tumours may also have been treatment-related according to the DS, although evidence for causality was not available (there was no other clear evidence of an effect on the ovary nor any indication of hormonal disturbance).

The increased incidence of astrocytomas in the top dose males was considered to be an incidental finding by the DS for several reasons: the historical control incidence was only exceeded by one animal, no other treatment-related changes in brain pathology were noted, and the brain of males had a relatively low level of exposure to penflufen and its metabolites compared to other tissues, as shown in toxicokinetic studies.

Finally, an increased incidence of histiocytic sarcomas was observed in the males. Treatment-related findings in the bone marrow, spleen, thymus and lymph nodes were not detected in any of the repeat dose studies (including the carcinogenicity study), so there was no evidence to support a MoA involving chronic injury in the haematopoietic system, and there was no evidence to support any alternative MoA. According to the DS, it is possible that these were incidental findings, but it is also plausible that they could indicate a very weak carcinogenic response to penflufen administration.

Mouse carcinogenicity study

In the mouse, a slightly increased incidence of hepatocellular carcinoma was seen in males. Given that hepatocellular carcinoma was extremely rare historically in the strain of mouse tested and that the liver is clearly a target organ for penflufen, the DS considered the small numbers of tumours seen in both males and females administered penflufen as possibly treatment-related. However, they also pointed out the lack of dose-related increase in benign tumours and the increased survival of mid and top dose males, which could be an alternative explanation for the increased tumour frequencies.

Mechanistic studies investigating the MoA for liver tumours

Several mechanistic studies have been conducted to investigate whether the increased liver tumours seen in rats and mice treated with penflufen are linked to activation of the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR). This MoA can potentially be considered qualitatively not relevant for humans.

The mechanistic studies included (1) an *in vitro* study in rat hepatocytes; (2) an *in vivo* study in female rats; (3) an *in vivo* study in male mice; and (4) an *in vitro* study in human hepatocytes. According to the DS, the results of the two *in vivo* studies were generally consistent with activation of CAR/PXR, with the exception of increased gene expression of Cyp1a1 observed in the rat, additionally indicating aryl hydrocarbon receptor (AhR) activation.

The *in vitro* study in human hepatocytes did not show increased cell proliferation and suggested PXR activation rather than CAR activation. However, the DS regarded the fact that cells from only one donor were used as a critical issue preventing reaching any firm conclusion about the relevance of the finding to the human population as a whole.

In summary, although the DS considered the CAR/PXR to provide a plausible explanation for the slightly increased incidence of liver tumours seen in some groups of penflufen treated animals, they concluded that a definitive conclusion is not possible on the basis of the available evidence because of several issues and uncertainties:

- Hepatocytes from only one human donor were investigated;
- Increased Cyp1a1 was observed in rats but no information is available on CYP1A1 in humans;
- The carcinogenic responses were weak;
- Sex-specific observations in carcinogenic response could not be explained by the mechanistic data (no studies were conducted with male rats or female mice);
- In rats, tumours were also seen in the ovary, brain and haematopoietic tissues. These are not associated with CAR activation.

Dossier submitter's conclusion on classification

The DS considered that since the increased tumour incidences seen in rats and mice could not be dismissed completely as being incidental or of no relevance to humans, no classification is not possible.

As increased tumour rates were seen in both rats and mice, a Category 1B classification could be considered. However, the DS preferred Category 2 on the basis of the following evidence:

- Penflufen is non-genotoxic;
- The increased tumour frequencies were slight, only just outside control ranges and they could have arisen by chance;
- A clear mechanistic basis for penflufen carcinogenicity is lacking;
- The increased frequencies of non-hepatic tumours were only evident in rats;
- Some of the increases were of benign tumours only.

Comments received during public consultation

Two MSCAs supported the dossier submitter's proposal for classification in Category 2. Another MSCA supported classification but did not specify which category they preferred.

Two MSCAs supported classification, but considered Category 1B more appropriate. In their argumentation, they referred to the occurrence of both carcinoma and sarcoma, occurrence of liver tumours in two species, the rarity of hepatocellular carcinomas in the mouse strain tested, premature deaths of the animals with histiocytic sarcomas and astrocytomas, and the occurrence of metastasis.

One manufacturing company submitted a position paper favouring no classification. They concluded that out of the tumours observed, only the hepatocellular adenoma in female rats could possibly be treatment-related, and that this effect resulted from a phenobarbital-like mechanism of action. The incidences of the other tumour types in the rat were similar to internal or external control data and did not show a dose-response relationship; taking into account the lack of genotoxic potential, these findings were regarded as incidental. The hepatocellular carcinomas in male mice were considered to be without any dose-effect relationship over a large range of dose levels, not associated with an increased incidence of pre-neoplastic changes and only marginally outside the historical control range.

Another manufacturing company also disagreed with the proposed carcinogenicity classification and highlighted the importance of penflufen for the wood protection market.

Assessment and comparison with the classification criteria

Rat carcinogenicity study

In this combined chronic toxicity and carcinogenicity study (IIA 5.5.2/1), 60 animals per sex per dose group were administered penflufen at dietary levels of up to 7000 ppm (288/399 mg/kg bw/d, m/f) for 2 years. Additional 20 animals/sex/dose were allocated for interim sacrifices. The non-neoplastic findings have been described in the STOT RE section. The most notable non-neoplastic finding was an increase in liver weight (by up to 30%) which was associated with hepatocellular hypertrophy. In addition, increased incidences of eosinophilic foci of cellular alteration was observed in females of all dose groups.

In females, treatment with 7000 ppm resulted in reduced body weight gain (by 18%). Increased survival was observed in both the 2000 ppm and 7000 ppm female groups (43/60 at both doses vs 29/60 in controls at scheduled kill).

Neoplastic findings are summarised in the table below. The findings discussed further are highlighted in grey.

Incidences of the neoplastic findings in the rat carcinogenicity study					
Dose (ppm)	**0**	**100**	**2000**	**7000**	**Historical control[a]**
Dose (mg/kg bw/d) m/f	0	4.0/5.6	79/113	288/399	
Number of animals (m, f)	60, 60	60, 60	60, 60	60, 60	50–60 per control group; 9–10 studies for each tumour type
Liver					
Males: Hepatocellular adenoma	1 (1.7%)	1 (1.7%)	0 (0%)	2 (3.3%)	Range: 0–3 (0–5%) Mean: 2.4%
Males: Hepatocellular carcinoma	1 (1.7%)	1 (1.7%)	0 (0%)	0 (0%)	
Females: Hepatocellular adenoma	0 (0%)	2 (3.3%)	5* (8.3%)	4 (6.7%)	Range: 0–3 (0–5%) Mean: 1.9%
Females: Hepatocellular carcinoma	0 (0%)	0 (0%)	1 (1.7%)	0 (0%)	
Ovary					
Tubulostromal adenoma	2 (3.3%)	1 (1.7%)	1 (1.7%)	7 (12%)	Range: 0–4 (0–6.7%) Mean: 2.6%
Tubulostromal adenocarcinoma	0 (0%)	1 (1.7%)	1 (1.7%)	0 (0%)	
Brain					
Males: Astrocytoma	1 (1.6%)	0 (0%)	0 (0%)	3 (5%)	Range: 0–2 (0–3.7%) Mean: 1.5%
Females: Astrocytoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	Range: 0 Mean: 0
Haematopoietic system					
Males: Histiocytic sarcoma	0 (0%)	3 (5%)	3 (5%)	5* (8%)	Range: 0–2 (0–3.3%) Mean: 1.5%
Females: Histiocytic sarcoma	3 (5%)	0 (0%)	0 (0%)	0 (0%)	Range: 0–4 (0–6.7%) Mean: 1.1%

* significantly different from control, $p \leq 0.05$

[a] The historical control data come from the same laboratory and strain and were compiled from studies commencing within the 7 years (2000–2006) preceding the beginning of the present study (in 2007).

Mouse carcinogenicity study

In the mouse carcinogenicity study (IIA 5.5.3/1), 50 animals per sex per dose group were administered penflufen at dietary levels of up to 6000 ppm (880/1101 mg/kg bw/d, m/f) for 18 months. The non-neoplastic findings have been described in the STOT RE section. Similarly to the rat, the main non-neoplastic finding was an increase in liver weight (by up to 24%) associated with hepatocellular hypertrophy.

No effect on body weight was noted in the treated groups. The survival of mid- and high-dose males was slightly increased compared to controls (high-dose 47/50, mid-dose 43/50, control 36/50).

Neoplastic findings are summarised in the table below. The findings discussed further are highlighted in grey.

Incidences of the neoplastic findings in the mouse carcinogenicity study					
Dose (ppm)	**0**	**100**	**1000**	**6000**	**Historical control[a]**
Dose (mg/kg bw/d) m/f	0	14.3/18.4	146/182	880/1101	
Number of animals (m, f)	48, 50	49, 50	49, 50	48, 50	50 per control group; 10 studies
Liver					
Males: Hepatocellular adenoma	1 (2%)	5 (10%)	1 (2%)	4 (8%)	Range: 0–4 (0–8%) Mean: 1.4%
Males: Hepatocellular carcinoma	1 (2%)	1 (2%)	3 (6%)	3 (6%)	Range: 0 Mean: 0
Females: Hepatocellular adenoma	1 (2%)	0 (0%)	1 (2%)	0 (0%)	Range: 0–2 (0–4%) Mean: 0.8%
Females: Hepatocellular carcinoma	0 (0%)	0 (0%)	0 (0%)	1 (2%)	Range: 0 Mean: 0

* significantly different from control, $p \leq 0.05$ (here none of the findings were statistically significant)

[a] The historical control data come from the same laboratory and strain and were compiled from studies commencing within the 7 years (2000–2006) preceding the beginning of the present study (in 2007).

Liver tumours – rat

The following table compares the incidences of liver tumours with incidences of liver hypertrophy and foci of cellular alteration in the rat carcinogenicity study.

Neoplastic and non-neoplastic liver findings in the rat carcinogenicity study								
	Males				**Females**			
Dose (ppm)	**0**	**100**	**2000**	**7000**	**0**	**100**	**2000**	**7000**
Dose (mg/kg bw/d)	0	4.0	79	288	0	5.6	113	399
Number examined	60	60	60	60	60	60	60	60

Rel. liver wt (% bw)	2.14	2.15	2.20	2.50** (+17%)	2.46	2.38	2.50	2.79** (+13%)
Hepatocellular hypertrophy	0	5*	21**	50**	0	0	22**	47**
Hepatocellular brown pigment, focal	0	1	9**	23**	0	0	18**	30**
Eosinophilic focus(i) of hepatocellular alteration	23	30	32	30	27	38	46**	39*
Hepatocellular adenoma	1	1	0	2	0	2	5*	4
Hepatocellular carcinoma	1	1	0	0	0	0	1	0

* significantly different from control, p ≤ 0.05; ** significantly different from control, p ≤ 0.01

The table shows that the incidence of altered foci correlated well with the incidence of adenoma. However, the correlation between the incidence of pre-neoplastic/neoplastic lesions and hypertrophy was considerably weaker in females and, interestingly, no increase in preneoplastic or neoplastic findings was observed in males despite a similar degree of hypertrophy.

There is no explanation for this sex difference. Still, the correlation between the adenoma incidence and the incidence of altered foci indicates a biologically plausible sequence of events, suggesting a weak treatment-related carcinogenic effect in female rats.

The DS considered that the findings in females could be attributed to increased survival. An assessment of this would have required information that would enable incidences of neoplastic and preneoplastic lesions to be related to time of death/sacrifice.

Liver tumours – mouse

The incidences of liver tumours are compared with incidences of liver hypertrophy in the mouse study in the table below. No increase in preneoplastic changes such as eosinophilic foci of hepatocellular alteration was detected in mice.

Neoplastic and selected non-neoplastic liver findings in the mouse carcinogenicity study								
	Males				**Females**			
Dose (ppm)	**0**	**100**	**1000**	**6000**	**0**	**100**	**1000**	**6000**
Dose (mg/kg bw/d)	0	14.3	146	880	0	18.4	182	1101
Number examined	48	49	49	48	50	50	50	50
Rel. liver wt (% bw)	4.48	4.39	4.57	5.39** (+20%)	5.32	5.40	5.56	6.60** (+24%)
Hepatocellular hypertrophy	0	13**	29**	46**	0	3	5*	31**
Hepatocellular adenoma	1	5	1	4	1	0	1	0

Hepatocellular carcinoma	1	1	3	3	0	0	0	1

* significantly different from control, p ≤ 0.05; ** significantly different from control, p ≤ 0.01

Although the dose-response relationship is not very clear and there is no statistical significance, the incidences of carcinomas in the mid and top dose males are relatively high considering their absence in the historical controls.

One hepatocellular carcinoma was also observed in top dose females, which might be perceived as a significant finding against the zero incidence in historical controls. On the other hand, one carcinoma appeared in control males in this study despite the absence of this finding in 10 previous studies. When additionally considering the lack of statistical significance, the probability that the single carcinoma in top dose females occurred by chance is relatively high. Therefore, hepatocellular carcinoma in females will not be considered further.

No increase in cell proliferation or benign tumours was observed in the present carcinogenicity study. However, increased hepatocellular proliferation was observed in male mice upon short-term penflufen administration in a mechanistic study (see below). Thus, these data are considered to provide indications of a possible carcinogenic effect in male mice.

Mode of action for liver tumours

The CAR-mediated MoA for rodent liver tumours consists of the following key events (KEs) (Elcombe *et al*., 2014; Peffer *et al*., 2018):

KE1: CAR activation
KE2: Altered gene expression specific to CAR activation
KE3: Cell proliferation
KE4: Clonal expansion leading to altered foci
KE5: Liver adenomas/carcinomas

Altered gene expression leads to several associative events, out of which the following ones have been considered as the most feasible to demonstrate as part of a regulatory dataset (Peffer *et al*., 2018):

AE1: Increased Cyp2b, Cyp3a enzyme activity and/or protein
AE2: Hepatocellular hypertrophy
AE3: Increased liver weight

<u>KE1 and KE2: CAR activation and altered gene expression</u>

Changes in expression of genes involved in phase I and phase II xenobiotic metabolism upon administration of penflufen have been investigated in two *in vivo* studies: one in female rats and one in male mice. Both studies employed a single dose, equal to the top dose in the carcinogenicity study. Phenobarbital was used as a positive control. The transcription profiles of Cyp enzymes in both species are summarised in the following table (for the full picture including phase II-related genes please see tables 21 and 22 in the CLH report).

Gene transcription in the liver of female rats and male mice after 7-day administration or penflufen or phenobarbital					
Gene transcripts	**Interpretation**	**Rat**		**Mouse**	
		Penflufen	**Phenob.**	**Penflufen**	**Phenob.**
Cyp1a1 (rat, mouse)	AhR activation	↑ 5.1-fold	No change	No change	↑ 1.7-fold

Cyp2b1 (rat) Cyp2b9 / Cyp2b10 (mouse)	CAR activation	↑ 6.5-fold	↑ 28-fold	No change / ↑ 17-fold	↑ 17-fold / ↑ 72-fold
Cyp3a3 (rat) Cyp3a11 (mouse)	CAR/PXR activation	↑ 11-fold	↑ 8.9-fold	↑ 2.0-fold	↑ 4.3-fold
Cyp4a1 (rat) Cyp4a10 (mouse)	PPARα activation	No change	No change	↑ 1.4-fold	No change

Penflufen induced Cyp2b-related gene expression in both rats and mice. The Cyp2b(9,10)/Cyp3a11 expression ratio was comparable between penflufen and phenobarbital in the mouse, but Cyp3a3 expression exceeded that of Cyp2b1 in the rat, in contrast to what was seen with phenobarbital.

In addition, Cyp1a1 gene expression was increased in the rat (not in the mouse), which might indicate activation of AhR.

AE1: Increased Cyp2b and Cyp3a enzyme activity

Induction of liver enzymes was another parameter measured in the two *in vivo* mechanistic studies. The results for Cyp activities are summarised in the table below.

Liver enzyme activities in female rats and male mice after 7-day administration of penflufen or phenobarbital					
Enzyme (class)	**Interpretation**	**Rat**		**Mouse**	
		Penflufen	**Phenob.**	**Penflufen**	**Phenob.**
EROD (Cyp1a)	AhR activation	No change	No change	↑ 1.7-fold	↑ 2.5-fold
PROD (Cyp2b)	CAR activation	↑ 3.7-fold	↑ 9.1-fold	↑ 7.7-fold	↑ 23-fold
BROD (Cyp2b/Cyp3a)	CAR/PXR activation	↑ 17-fold	↑ 39-fold	↑ 58-fold	↑ 163-fold
Lauric acid hydroxylation (Cyp4a)	PPARα activation	No change	No change	No change	No change

EROD = ethoxyresorufin *O*-deethylation; PROD = pentoxyresorufin *O*-depentylation; BROD = benzyloxyresorufin *O*-debenzylation

The liver enzyme induction profile of penflufen is most consistent with CAR/PXR activation, and the Cyp2b/Cyp3a induction ratio is comparable to that seen after treatment with phenobarbital. Interestingly, the increased gene expression of Cyp1a1 in the rat did not translate into increased EROD activity. This reduces somewhat the concern for AhR activation, given further that in mice there was a slight increase in EROD activity without increased gene expression of Cyp1a1.

AE2 and AE3: Hepatocellular hypertrophy and increased liver weight

Hepatocellular hypertrophy, accompanied by liver weight increases at higher doses, has been consistently observed in both rats and mice in several studies including the carcinogenicity studies. As already mentioned, the degree of hypertrophy was comparable between males and females, and yet the increase in liver tumours was limited to a single sex in both species. The cause of this sex difference is not known.

KE3: Cell proliferation

Cell proliferation was measured as a BrdU labelling index in the two *in vivo* mechanistic studies. The results are summarised in the following table.

Hepatocellular proliferation in female rats or male mice after 7-day administration of penflufen or phenobarbital				
	Rat		**Mouse**	
	Penflufen	**Phenobarb.**	**Penflufen**	**Phenobarb.**
Cell proliferation in the centrilobular area	↑ 1.6-fold	↑ 1.5-fold	↑ 1.6-fold	↑ 3-fold

Increased cell proliferation was observed with both substances in both species, although statistical significance has not been reached in any of the groups.

KE4: Clonal expansion leading to altered foci

An increase in this pre-neoplastic lesion was only observed in female rats and it correlated well with the increase in adenomas. Foci of cellular alteration have not been observed in the mice. However, altered foci at tumorigenic doses are not observed with all CAR activators, so demonstration of this key event is not considered critical (Peffer *et al*., 2018).

KE5: Liver adenomas/carcinomas

As mentioned earlier, the increase in incidence of carcinomas in male mice was weak relative to concurrent controls, and did not show a clear dose-response relationship. It is mainly the comparison with the historical control incidence that raises a concern. Thus, the CAR/PXR activation did not translate into an unequivocal increase in liver tumours in the mouse.

In the female rat, the increase in liver tumours is clearer, although the reasons for the weak dose-response relationship in the females and the lack of carcinogenic effect in the males are not known.

Exclusion of alternative MoAs

Alternative MoAs are discussed in the following table.

Mode of action	**Data relating to penflufen**
Genotoxicity	Negative data in standard tests
PPARα activation	Cyp4a gene transcription and enzyme induction not increased in the rat and mouse
AhR activation	Cyp1a1 expression increased in the rat, but did not translate into increased EROD activity. No increase in Cyp1a expression and slightly increased EROD activity in the mouse, but the increase was lower than that caused by phenobarbital.
Cytotoxicity	No histopathological evidence of necrosis, fibrosis or inflammation; no increase in serum ALT or AST
Porphyria, iron overload	Hepatocellular brown pigment of unknown nature was observed in the rat carcinogenicity study in mid and top dose males and females. However, there was no indication of increased breakdown of red blood cells nor any evidence of hepatocellular necrosis.

Estrogenic activity	There was a slight increase in tubulostromal hyperplasia and tubulostromal adenoma in female rats in the two-year study, but no effects were seen in the ovaries in any of the repeat dose studies of shorter duration to indicate any hormonal disturbances. However, no studies specifically investigating estrogenic activity (e.g., measurements of hormone levels, *in vitro* assays) are available.
Immunosuppression	In the two-year study in male rats there was an increased incidence of histiocytic sarcoma, which is an immune cell malignancy. However, no changes in the immune system or immune cells were detected in any of the shorter term studies or in a 4-week immunotoxicity study.

Evidence in humans

The *in vitro* study in human hepatocytes used cells from one female donor. Summary of the *in vitro* human study is provided in the following table together with results from the *in vitro* rat study for comparison.

***In vitro* studies in human and rat hepatocytes**				
	Human		**Rat**	
	Penflufen	**Phenobarb.**	**Penflufen**	**Phenobarb.**
Concentrations tested (µM)	0.1–30	10–1000	0.1–100	10–1000
Cell proliferation (by BrdU incorp.)	No increase	No increase	↑ max. 1.7-fold (up to 3 µM)	↑ max. 1.8-fold (from 10 µM)
PROD activity (Cyp2b)	No change	↑ 2.6-fold (only at 1000 µM)	↑ max. 5-fold (at 0.1 µM)	↑ max. 10-fold (from 100 µM)
BROD activity (Cyp2b/Cyp3a)	↑ max. 1.5-fold (at 3 µM)	↑ max. 5-fold (at 1000 µM)	↑ max. 1.8-fold (at 30 µM)	↑ max. 5.7-fold (from 100 µM)
BQ activity (Cyp 3a)	↑ max. 2-fold (at 10 µM)	↑ max. 3.3-fold (at 1000 µM)	↑ max. 2.4-fold (at 100 µM)	↑ max. 8.4-fold (at 1000 µM)

PROD = pentoxyresorufin *O*-depentylation; BROD = benzyloxyresorufin *O*-debenzylation; BQ = benzyloxyquinoline *O*-debenzylation

Neither penflufen nor phenobarbital stimulated proliferation of human hepatocytes, whereas the positive control (epidermal growth factor) produced a 9-fold increase in replicative DNA synthesis.

As to enzyme induction, penflufen did not increase PROD (Cyp2b) activity in human hepatocytes. BROD (Cyp2b/Cyp3a) activity was only increased at one or two concentrations without a clear dose-response relationship while BQ (Cyp3a) activity was clearly increased at higher concentrations. This pattern indicates PXR activation rather than CAR activation in human hepatocytes.

Nevertheless, the key observation with regard to the proposed MoA is the presence of increased cell proliferation in the rat cells versus lack thereof in human hepatocytes.

The limitations to interpretation arising from the fact that cells from only one human donor were used is acknowledged by RAC. Although there is currently no consensus on the minimum number of human donors to be used in a study of this kind, RAC was provided with studies using more than one donor in other cases. Therefore, although RAC does not disregard the current study, using only one donor is considered to be a weakness limiting the interpretation and this fact is further considered in the weight of evidence assessment.

No studies with animals containing humanised CAR/PXR were available.

Conclusion on the MoA of liver tumours

The critical key events of the CAR-mediated MoA for liver tumours have been shown to occur in both the rat and the mouse:

- Altered gene expression specific to CAR activation
- Increased cell proliferation
- Liver tumours

The proposed MoA is further supported by the observation of altered foci in the female rat (key event 4) and by liver hypertrophy and induction of Cyp2b/Cyp3a (associative events) in both species.

Based on the data available, CAR or CAR/PXR activation seems a plausible mechanism to explain the slightly increased incidence of liver tumours in some groups of treated rodents. On the other hand, RAC notes that the investigations into this MoA have not been as extensive as for other potential CAR activators previously evaluated by RAC, and that there were only a limited number of investigations to exclude other MoAs that could also potentially explain the liver tumours.

The CAR-mediated MoA for liver tumours can potentially be considered as qualitatively not relevant for humans (Elcombe *et al*., 2014). That holds particularly true when there would be qualitative differences between humans and rodents in the prerequisite step for tumour formation, *i.e.* DNA replication. For penflufen this is possibly the case, given a lack of increased cell proliferation in an *in vitro* study with human hepatocytes, in contrast to a positive response in rat hepatocytes. The evidence is however considered too limited (cells from a single donor only, no studies with animals containing humanised CAR/PXR) to draw firm conclusions.

In summary, the data available seem most consistent with CAR or CAR/PXR activation. However, uncertainty remains regarding exclusion of alternative MoAs and human relevance.

Ovarian tubulostromal adenomas

The incidence of ovarian tubulostromal adenomas was increased above the historical control range in the top dose rats, but without statistical significance and without a convincing increase in hyperplasia. The data on tubulostromal hyperplasia and tubulostromal tumours are presented in the following table.

Incidences of ovarian tubulostromal hyperplasia and tumours in the rat carcinogenicity study					
Dose (ppm)	**0**	**100**	**2000**	**7000**	**Historical control**
Dose (mg/kg bw/d)	0	5.6	113	399	
Number examined	60	60	60	60	50–60 per control group; 10 studies
Tubulostromal hyperplasia, focal	3 (5%)	4 (6.7%)	1 (1.7%)	7 (12%)	Range: 0–15 (0–25%) Mean: 12%
Tubulostromal hyperplasia, focal: minimal to slight	2	2	0	5	
Tubulostromal hyperplasia, focal: moderate to marked	1	2	1	2	

Tubulostromal adenoma	2 (3.3%)	1 (1.7%)	1 (1.7%)	7 (12%)	Range: 0–4 (0–6.7%) Mean: 2.6%
Tubulostromal adenocarcinoma	0	1	1	0	

Although the findings were not statistically significant and there was no increase in moderate or marked hyperplasia, a weak treatment-related effect cannot be excluded.

Brain astrocytomas

A statistically nonsignificant increase in brain astrocytomas was observed in the top dose rat males (1, 0, 0, 3 corresponding to 1.6%, 0%, 0%, 5% in the control, low, mid and high dose group, respectively). It exceeded the historical control range by one animal (HCD 0–2 cases per group, corresponding to 0–3.7%; mean 1.5%). All 3 top dose males with astrocytoma died prematurely during the study while the 1 control male was found to have astrocytoma at the terminal kill. No preneoplastic lesions or benign tumours were noted.

In view of the reduced survival of animals with tumours, RAC considers the brain astrocytomas as possibly related to treatment, but the concern is lessened by the relatively low incidence compared to the concurrent control, by the absence of preneoplastic lesions or benign tumours in any of the dose groups, and by the absence of brain tumours in females despite higher concentration of the substance in the brains of females (demonstrated in ADME studies).

Histiocytic sarcomas

An increase in histiocytic sarcomas, which was statistically significant and exceeded the historical control range, was observed in the top dose males, although the dose-response relationship was not very clear (incidences 0, 3, 3, 5 corresponding to 0%, 5%, 5%, 8% in the control, low, mid and top dose group respectively; HCD 0–3.3%, mean 1.5%). The 3 animals with tumours in the mid dose group and 2 out of 5 animals in the top dose group died prematurely. There were no histiocytic sarcomas in the chronic phase of the study (1 year, 20 animals/sex/dose). Many of the affected animals had metastasis, which is a feature typical for this kind of tumour. No treatment-related findings were identified in the bone marrow, spleen, thymus or lymph nodes.

Although females had a higher background incidence of this tumour (HCD range 0–6.7%, mean 1.1%) and the incidence in the concurrent control was 5% (3 cases), no histiocytic sarcoma was found in the treated groups. No increase was seen in mice, despite the generally higher susceptibility of this species to histiocytic sarcomas (Greaves, 2012).

Conclusion on classification

In the absence of human data, Category 1A is not applicable.

Category 1B is appropriate if there is sufficient evidence of carcinogenicity in animals whereas Category 2 is intended for cases where the evidence for carcinogenicity is limited. In the discussion below, in the references to "sufficient evidence" and "limited evidence", these terms are as defined in Annex I, 3.6.2.2.3 (b) of the CLP Regulation. According to the CLP regulation, carcinogenicity classification should be based on a weight of evidence approach and many factors increasing or decreasing the concern should be taken into account.

The histiocytic sarcomas in male rats raise concern for carcinogenicity due to their malignancy, reduced survival of some of the affected animals and a statistically significant increase above HCD. On the other hand, RAC notes the weak dose-response relationship, absence of any histiocytic sarcomas in treated females despite a higher background incidence in this sex, and absence of this finding in the mouse, a species generally more susceptible than rats to the

induction of histiocytic sarcomas. Thus, RAC considers the histiocytic sarcomas to amount to only limited evidence of carcinogenicity.

The brain astrocytomas in male rats raise concern by their malignant nature and reduced survival of the affected animals. On the other hand, the lack of statistical significance, the increase only slightly above HCD, occurrence of the tumours in only one sex and the absence of preneoplastic lesions reduce the concern. Therefore, RAC considers the astrocytomas to amount to limited evidence of carcinogenicity.

The ovarian tubulostromal adenomas in the rat are considered to provide some support to classification but are not sufficient to trigger classification on their own due to the lack of statistical significance, lack of preneoplastic lesions, their benign nature and occurrence in only one species.

The liver tumours (adenomas in female rats and carcinomas in male mice) provide some support to classification, but are not sufficient to trigger classification on their own due to the weak carcinogenic response (lack of statistical significance, lack of dose-response relationship) and their benign nature (adenoma). Further, the available MoA information, albeit not conclusive, does not indicate specific concern for humans.

As increased tumour incidences were observed in several tissues and in two species, Category 1B has to be considered. However, taking into account the sex- and species-specificity of the malignant tumours, lack of statistical significance and/or weak dose-response relationships, lack of any indication of genotoxicity in mutagenicity tests, and MoA information, RAC considers that the findings do not amount to "sufficient evidence" of carcinogenicity.

RAC considers the increased incidences of histiocytic sarcomas, astrocytomas, ovarian tubulostromal adenomas and hepatocellular adenomas in the rat and hepatocellular carcinomas in the mouse to collectively amount to limited evidence of carcinogenicity, and therefore RAC agrees with the dossier submitter's conclusion that **classification as Carc. 2** is warranted for penflufen.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The reproductive toxicity of penflufen has been investigated in a guideline-compliant two-generation study in the rat and in guideline-compliant prenatal developmental toxicity (PNDT) studies in the rat and the rabbit.

There was no evidence of a specific effect on fertility, sexual function or reproduction in the two-generation study, according to the DS. In the high-dose group, slight reductions in the mean litter size and reductions in pup bodyweight during lactation were considered secondary to maternal toxicity.

There were no adverse effects on development in the rat PNDT study. In rabbits, malformations were reported in all dose groups, but without a dose-response relationship.

Classification for adverse effects on or via lactation was not addressed by the DS.

The DS considered the available data conclusive but not meeting the criteria for classification for reproductive toxicity.

Comments received during public consultation

One MSCA agreed with the DS that there were no effects on fertility or development.

Another MSCA supported no classification for fertility, but proposed classification for developmental effects based on the malformations seen in the rabbit PNDT study. They considered all the malformations to have the same developmental aetiology consisting of vascular disruption during embryogenesis. The MSCA's proposal was for Category 2 due to the absence of malformations in the rat study. The DS in their response emphasised the lack of dose response relationship and especially the limited number of malformations in the top dose group.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

Two-generation study

The two-generation study is summarised in the following table.

Two-generation study		
Type of study; Reference (DAR); Year	**Method**	**Observations**
2-generation reproductive study, dietary, rat IIA 5.6.1/1 Year: 2009	OECD TG 416 GLP Doses: 0, 200, 1000, 4000 ppm; corresponding to 0, 12/15, 58/71, 252/293 mg/kg bw/d (m/f) 30 parental animals/sex/dose	Parental findings 4000 ppm (252/293 mg/kg bw/d), both sexes and generations: • ↓ bw (by up to 10%) • ↑ liver wt (relative by approx. 20%), hepatocellular hypertrophy (minimal) ≤ 1000 ppm (58/71 mg/kg bw/d): • ↑ liver wt (F0 m) Reproductive findings 4000 ppm (252/293 mg/kg bw/d): • ↓ mean number of pups delivered (F0 by 13%; F1 by 11%) ≤ 1000 ppm (58/71 mg/kg bw/d): No effects Offspring findings 4000 ppm (252/293 mg/kg bw/d), both generations: • ↓ bw (no difference on PND0; ↓ by approx. 7% on PND7 and by approx. 11% on PND21) • ↓ spleen wt (relative by approx. 14%/11% F1/F2) • ↑ time to vaginal opening (by 12%/8% F1/F2) ≤ 1000 ppm (58/71 mg/kg bw/d): No effects

Reduced litter size was observed at the top dose in both generations. The reductions were not statistically significant but exceeded HCD ranges. The data on litter size and related parameters are shown in the following table.

Two-generation study: litter size and related parameters					
Dose (ppm)	**0**	**200**	**1000**	**4000**	**HCD**[b]
F0/F1					
Mean litter size on day 0	10.6	10.1	10.6	9.2	17 studies 9.8–12.8
Mean no. of implantation sites	11.2	10.8	10.6	10.3	
Post-implantation loss[a] (%)	5.8	7.6	5.9	9.9	
F1/F2					
Mean litter size on day 0	10.4	10.1	10.7	9.3	8 studies 10.4–10.9
Mean no. of implantation sites	10.7	10.9	11.1	10.0	
Post-implantation loss[a] (%)	3.3	8.3	4.3	8.1	

[a] calculated by RAC from the 'birth index' reported by the DS; the 'birth index' is defined as no. of pups born per litter/no. of implantation sites per litter x 100, so post-implantation loss + birth index yields 100%; statistical analysis not conducted for post-implantation loss

[b] The historical control data were from the same laboratory and strain and from studies performed within 5 years of the current study.

The reduction in litter size at 4000 ppm was observed in the preliminary one-generation study and in both generations of the main study. On the other hand, no reduction in litter size was observed at 7000 ppm in the preliminary study, so interpretation of the reduced litter size at 4000 ppm is not straightforward. Taking these data together, and considering that the reduced litter size at 4000 ppm was not statistically significant, RAC does not consider the effect sufficient to trigger classification.

RAC notes the limited maternal toxicity at 7000 ppm in the preliminary study (reduced body weight by up to 9%, no clinical signs of toxicity), and that rats tolerated 7000 ppm in the 2-year carcinogenicity study with only a reduction in body weight gain of 18% in the females. This indicates that this dose could probably have been chosen for the main two-generation study without exceeding the MTD, and that 4000 ppm as the top dose was too low to fully inform about the potential reproductive toxicity of the substance.

Statistically significant reductions in body weight of the top dose pups starting from PND7 were observed in both generations. The mean pup body weight was unchanged on PND0, but then it was reduced by approximately 8%, 9%, and 11% on PND7, PND14, and PND21 respectively in the F1 generation. A comparable reduction was seen in F2 pups (0%, 6%, 8%, and 10% on PND0/7/14/21, respectively). This might indicate an effect on or via lactation. However, classification for adverse effects on or via lactation is not considered justified due to the low magnitude of the effect and the lack of further data (e.g., on concentration of penflufen and its metabolites in the milk).

Vaginal opening was statistically significantly delayed at the top dose in F1 offspring (39.6 days vs 35.5 days in controls) and non-significantly (39.8 days vs 36.7 days) in the F2 offspring. This may reflect a general developmental delay associated with reduced pup body weight.

Conclusion on classification

RAC agrees with the DS that the available data **do not warrant classification for adverse effects on sexual function and fertility.** RAC however notes that the available data might not fully inform on the reproductive toxicity of penflufen, due to too low dosing.

Adverse effects on development

The prenatal developmental toxicity studies are summarized in the following table.

Prenatal developmental toxicity studies		
Type of study; Reference (DAR); Year	**Method**	**Observations**
PNDT study, gavage, rat IIA 6.6.2/1 Year: 2008	OECD TG 414 GLP Doses: 0, 30, 100, 300 mg/kg bw/d Dosing GD 6-20 23 females/dose	Maternal findings 300 mg/kg bw/d: • ↓ bw gain (GD 6-21 by 13%) • ↑ liver wt ≤ 100 mg/kg bw/d: No effects Developmental findings ≤ 300 mg/kg bw/d: No effects
PNDT study, gavage, rabbit IIA 6.6.3/1 Year: 2008	OECD TG 414 GLP Doses: 0, 30, 100, 600 mg/kg bw/d Dosing GD 6-28 23 females/dose	Maternal findings 600 mg/kg bw/d: • 1 animal killed for humane reasons on GD 25 (no faeces, severe bw loss; no macroscopic abnormalities at necropsy) • ↓ food consumption (by approx. 20% GD 6-22) ≤ 100 mg/kg bw/d: No effects Developmental findings The developmental findings are summarised in a separate table below

Rat PNDT study

The rat PNDT study was clearly negative regarding developmental toxicity, but the maternal toxicity at the top dose was limited to a mild reduction in body weight gain. The DAR states that the dose levels were chosen on the basis of a range-finding study, in which marked maternal toxicity, including mortality, was seen at 1000 mg/kg bw/d. This indicates that the MTD lies between 300 and 1000 mg/kg bw/d and a higher top dose might have been appropriate.

Rabbit PNDT study

The pregnancy and foetal data are summarized in the following table.

PNDT study in rabbits				
Parameter	**Penflufen (mg/kg bw/d)**			
	0	**30**	**100**	**600**
Pregnant females (out of 23)	22	23	23	19

Post-implantation loss (%)	10.0	5.0	15.8	11.5
Total no. of early resorptions (number per dam)	7 (0.3)	3 (0.1)	23 (1.1)	7 (0.4)
Total no. of dead foetuses (% per litter)	12 (5.3%)	7 (3.1%)	12 (4.9%)	16 (8.0%)
Total no. of foetuses examined	191	218	187	167
No. of malformed foetuses (litters)	3 (3)	7 (5)	5 (5)	2 (2)

The malformations are specified below.

Description of the malformations in the rabbit PNDT study	
Dose (mg/kg bw/d)	**Description**
0	2 foetuses with various malformations of the ribs and vertebrae 1 foetus with multiple malformations; forelimb amelia, diaphragmatic hernia, absent forelimb bones
30	1 foetus with multiple malformations; gastroschisis, absent kidneys and skeletal (ribs, vertebrae, sternebrae, limbs) 3 foetuses with various skeletal malformations of the ribs and/or vertebrae and/or sternebrae 1 foetus with multiple malformations; gastroschisis, anasarca, short snout, malrotated forepaw and skeletal (sternebrae) 1 foetus with absent right atrioventricular valve 1 foetus with diaphragmatic hernia
100	1 foetus with multiple malformations; micrognathia, cleft palate, short trunk, bent tail, malpositioned digits on forepaws and skeletal (small mandible, split/bent palatine/clavicle) 1 foetus with cardiovascular malformations (small left atrium, enlarged right atrium, dilated ascending aorta, enlarged right ventricle, ventricular septum defect in median region, small left ventricle) and skeletal (sternebrae) malformations 1 foetus with hydropericardium 2 foetuses with skeletal (rib and vertebrae) malformations
600	1 foetus with multiple cardiovascular (dilated aortic arch and ascending aorta, pulmonary trunk atresia, small right ventricle, enlarged left ventricle) malformations 1 foetus with omphalocele

Firstly, there was a marginal increase in dead foetuses in the top dose group which according to the CLH report exceeded historical control range. The DS explained that this increase was largely attributable to a single female with 6 dead foetuses.

The increase in the number of malformations was not dose-related and, importantly, there was no increase in post-implantation loss. However, the slight increase in the number of dead foetuses in the top dose group introduces uncertainty to this conclusion.

Malformations of limbs, ribs and vertebrae as well as diaphragmatic hernia were present in the control group, hence the low incidences of these findings in the treated groups are considered incidental.

Two cases of gastroschisis were present at 30 mg/kg bw/d, but not at higher doses, and omphalocele was observed at the top dose of 600 mg/kg bw/d. Both findings were reported to exceed historical control data (in 10 studies within 7 years of the current study, 1 case of gastroschisis and no case of omphalocele). However, due to the lack of a dose-response relationship for gastroschisis and the low (single) incidence of omphalocele these findings are not considered sufficiently clear evidence of developmental toxicity to lead to classification.

One, 2, and 1 foetus with cardiovascular malformations were observed in the low, mid and high dose groups, respectively, while no cardiovascular malformations were found in the control foetuses. Nevertheless, in the absence of a dose-response relationship the cardiovascular malformations are not regarded as sufficiently clear evidence of developmental toxicity for classification.

Overall, RAC does not consider the rabbit study to provide any convincing evidence of a treatment-related developmental effect.

Conclusion on classification

As no convincing evidence of an adverse developmental effect has been found in the available studies, RAC concurs with the dossier submitter's conclusion that **no classification for adverse effects on development is warranted**.

Adverse effects on or via lactation

The possibility of classification for adverse effects on or via lactation was not addressed in the CLH report. Although the reductions in postnatal growth (reduced pup weight by up to 11% at weaning) observed in the 2-generation rat study might indicate an effect on or via lactation, classification is not considered justified due to the low magnitude of the effect and lack of further data (e.g., on concentration of penflufen and its metabolites in the milk).

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed to classify penflufen as Aquatic Acute 1 and Chronic 1 both with acute and chronic M-factors of 1. The substance was not rapidly degradable and it had no potential to bioaccumulate. The lowest acute toxicity value was a 96h LC_{50} of 0.103 mg/L for *Cyprinus carpio* (Common carp). On this basis penflufen was classified as Aquatic Acute 1 with an M-factor of 1 (range $0.1 < LC_{50} \leq 1$ mg/L). The lowest chronic value was a 35-days NOEC for *Pimephales promelas* (Fathead minnow) of 0.0234 mg/L. Given this was in the range 0.01 to 0.1 mg/L and the substance is considered non-rapidly degradable, penflufen should be classified as Aquatic Chronic 1 with an M-factor of 1 (range $0.01 < NOEC \leq 0.1$ mg/L).

Degradation

No significant hydrolysis was observed in an aqueous hydrolysis study (GLP, OECD TG 111) at 50 °C and at pHs 4, 7 and 9. Penflufen was thus considered hydrolytically stable.

Penflufen was susceptible to limited photodegradation. There were two aqueous photolysis studies available following GLP and US EPA Guideline Subdivision N, Series 161-2 and EU Council Directive 91/414/EEC, section 2, sub section 2.9.2 and SETAC procedures. In the first study, in a sterile aqueous buffer solution a number of degradants were observed at low levels. Mineralisation was low, the DT_{50} at 38.03 °N (Athens, Greece) was calculated to be 130.6 days in June and at 51.3 °N (London, UK) 163.6 days in July. In the second study, which used sterile natural river water from the Rhine, up to 15 degradants were observed at low levels. Mineralisation was low, the DT_{50} at 38.03 °N (Athens, Greece) was calculated to be 26.2 to 33.1 days in June and at 51.3 °N (London, UK), 32.7 to 41.4 days in July. In a GLP study performed following ECETOC methods, DT_{50} = 210 to 293 days and DT_{50} = 210 to 270 days at 50°N (Germany) in spring/summer sunlight were estimated using 2 different models, respectively.

No ready biodegradation study was available. In a GLP water-sediment study (OECD TG 308) using two aerobic systems, penflufen dissipated from the water phase to the sediment phase via partitioning with limited degradation in both phases. The degradation product penflufen-3-hydroxy-butyl (M01) was observed in both water and sediment at maxima of 10.7% AR in water and 2.1% AR in sediment at day 120. Minimum mineralisation was observed with a maximum of 3.2% AR after 120 days. Subsequent kinetic assessment derived a single first order geometric mean whole system DT_{50} of 221 days.

Aquatic toxicity data for identified degradants was presented in the CLH report showing that the degradants were less toxic than the parent substance. Data on degradants was not needed to assess rapid degradability of penflufen and thus was not considered further for classification of penflufen.

Overall, the degradation information did not provide sufficient data to show that penflufen was ultimately degraded with 28 days or transformed to non classifiable products. Consequently, penflufen was considered not rapidly degradable for the purpose of classification and labelling.

Bioaccumulation

In a fish BCF study performed according to GLP and OECD TG 305, the normalised (6% lipid content) whole fish steady state BCF was 12 L/kg ww. Whole fish kinetic BCFs based on Total Radioactive Residues were 100 to 103 L/kg. The log K_{OW} value was 3.3 at pH 4, pH 7 and pH 10. The whole fish BCF for penflufen is below the CLP trigger of ≥ 500 intended to identify substances with a potential to bioconcentrate. In addition the log K_{OW} is below the CLP trigger value of ≥ 4. Therefore, penflufen is not considered a bioaccumulative substance.

Aquatic toxicity

A summary of available valid information on the aquatic toxicity of penflufen is presented in the Table below.

***Table**. Summary of valid relevant information on aquatic toxicity of penflufen*

Guideline / GLP status	Species	Endpoint	Exposure		Results	
			Design	**Duration**	**Endpoint**	**Toxicity (mg/L)**
Acute toxicity to fish OECD TG 203, GLP, purity: 95.6%	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	0.31 (mm) [1] measured concentrations 88 to 104% of nominal
Acute toxicity to fish OECD TG 203, GLP, purity: 95.6%	Bluegill Sunfish (*Lepomis macrochirus*)	Mortality	Static	96 hours	LC_{50}	0.45 (mm) [1] measured concentrations 100 to 111% of nominal
Acute toxicity to fish OECD TG 203, GLP, purity: 95.6%	Fathead Minnow (*Pimephales promelas*)	Mortality	Static	96 hours	LC_{50}	0.116 (mm) [1] measured concentrations 85-92% of nominal
Acute toxicity to fish OECD TG 203, GLP, purity: 95.6%	**Common carp (*Cyprinus carpio*)**	**Mortality**	**Static**	**96 hours**	**LC_{50}**	**0.103 (mm) [1]** measured concentrations 98-128% of nominal
Acute toxicity to fish OECD TG 203, GLP, purity: 95.6%	Sheepshead Minnow (*Cyprinodon variegatus*)	Mortality	Static	96 hours	LC_{50}	1.15 (mm) [1] measured concentrations 82-96% of nominal
Fish Early Life-Stage (FELS) toxicity OECD TG 210, GLP, purity: 95.6%	**Fathead Minnow (*Pimephales promelas*)**	**Time to hatch, hatching success, survival and growth (length, wet weight and dry weight)**	**Flow-through**	**35 days**	**NOEC**	**0.0234 (mm) [1] for length** 0.0476 (mm) for survival, weight and morphological/ behavioural effects measured concentrations 93-99% of nominal
Daphnia sp Acute Immobilisation OECD TG 202 GLP, purity: 95.6%	*Daphnia magna*	Acute	Static	48 hours	EC_{50}	>4.66 (mm) [1] [2] 40% immobilisation mean measured 93-99% of nominal

Acute toxicity OECD TG 202, GLP, purity: 95.6%	Crayfish (*Procambarus clarkii*)	Acute	Static	96 hours	EC_{50}	>4.5 (mm) [1] [2] no effects mean measured 90-101% of nominal
Acute toxicity US EPA OPPTS 850.1025, GLP, purity:95.6%	Oyster (*Crassostrea virginica*)	Acute	Flow-through	96 hours	EC_{50}	1.3 (mm) [1] shell growth mean measured 56-74% of nominal
Acute toxicity US EPA OPPTS 850.1035, GLP, purity: 95.6%	Mysid Shrimp (*Americamysis bahia*)	Acute	Flow-through	96 hours	LC_{50}	2.5 (mm) [1] mean measured - 120-145% [2] (lowest conc.) -94-112% of nominal (other conc.)
Daphnia magna Reproduction OECD TG 211, GLP, purity: 95.6%	*Daphnia magna*	Survival; reproduction; growth	Semi-static	21 days	NOEC	1.53 (mm) [1] no effects at highest conc. mean measured 95-106% of nominal
Freshwater Algal Growth Inhibition OECD TG 201, GLP, purity: 95.6%	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	E_rC_{50} NOE_rC	>5.1 (mm) [1] [2] 0.52 (mm) mean measured 79-102% of nominal
Lemna sp. Growth Inhibition Test OECD TG 221, GLP, purity: 95.6%	*Lemna gibba*	Growth	Semi-static	7 days	$E_rC_{50(frond\ number)}$ $E_rC_{50(dry\ weight)}$ $NOE_rC_{(frond\ number)}$ $NOE_rC_{(dry\ weight)}$	>4.7 (mm) [1] [2] >4.7 (mm) 2.4 (mm) ≥4.7 (mm)

[1] solvent DMF used; [2] Due to limited solubility of penflufen, no higher concentration could be tested (DAR, Volume 3, B.9 August 2011); * formerly *Selenastrum capricornutum*; data that drives the classification in bold.

Acute toxicity

There are five acute toxicity studies following GLP and OECD TG 203 available for penflufen. The lowest value is a 96h LC_{50} of 0.103 mg/L for *Cyprinus carpio*.

For invertebrates, there are studies on *Daphnia*, freshwater crayfish (*Procambarus clarkii*), marine Eastern Oyster (*Crassostrea virginica*) and a marine Mysid (*Americamysis bahia*).

In the *Daphnia* study, the effects were observed at the highest exposure concentration with 40% immobilisation. The study 48h LC_{50} was > 4.66 mg/L. Due to limited solubility of penflufen, no higher concentration could be tested.

In the study on freshwater crayfish (*Procambarus clarkia*), observations of sub-lethal effects and mortality were recorded. No mortality/effects were seen at the highest test concentration and the study 96h EC_{50} was > 4.5 mg/L. Due to limited solubility of penflufen, no higher concentration could be tested.

A study on marine Eastern Oysters (*Crassostrea virginica*) recorded mortality and shell deposition endpoints. Based on shell growth, the 96h EC_{50} was 1.3 mg/L. In the marine Mysid *Americamysis bahia* study, the 96h LC_{50} based on mortality was 2.5 mg/L.

11% inhibition of growth was observed at the highest exposure concentration 5.14 mg/L in an algae growth inhibition study on *Pseudokirchneriella subcapitata*. The 72h E_rC_{50} was thus > 5.1 mg/L. The highest level tested was the functional limit of solubility in the test system.

The study endpoints were frond number, frond yield, biomass, growth rate and dry weight in a 7-day *Lemna gibba* study. The highest concentration tested was at the limit of solubility of the test system. Based on 10% inhibition observed at the highest exposure concentration 4.7 mg/L, the study 7d E_rC_{50} (frond number) was > 4.7 mg/L. Similarly, 6.6% inhibition was observed at the highest exposure concentration for the growth rate (dry weight) endpoint, so the study 7d E_rC_{50} (dry weight) was also > 4.7 mg/L. The lowest growth rate 7d NOE_rC was based on frond number at 2.4 mg a.s./l, based on mean measured.

The lowest acute aquatic toxicity value is a 96h LC_{50} of 0.103 mg/L for *Cyprinus carpio*.

Chronic toxicity

There were one chronic toxicity study on fish available. In the Fish Early Life-Stage (FELS) (OECD TG 210, GLP) with *Pimephales promelas*, time to hatch, hatching success, survival and growth (length and dry weight) were followed. The most sensitive endpoint was fish growth (length) where the 35d NOEC was determined to be 0.0234 mg/L.

A chronic toxicity study to *Daphnia magna* assessed survival, reproduction, length and weight. No significant effects were observed for any parameter. The study 21d NOEC was 1.53 mg/L reflecting the highest exposure concentration.

11% inhibition of growth was observed at the highest exposure concentration 5.14 mg/L in a algae growth inhibition study on Pseudokirchneriella subcapitata. The 72h NOE_rC was 0.52 mg/L.

The study endpoints were frond number, frond yield, biomass, growth rate and dry weight in a 7-day *Lemna gibba* study. The lowest growth rate 7d NOE_rC was based on frond number at 2.4 mg/L based on mean measured.

The lowest chronic aquatic toxicity value is a 35d NOEC of 0.0234 mg/L for *Pimephales promelas*.

Comments received during public consultation

Five MSCAs supported the DS proposal. An MSCA made a comment concerning the OECD TG 308 water simulation study mineralisation percentage. The DS agreed that there was a typographical error. The MSCA also proposed considering available data from a screening test following OECD TG 301C (2015) currently missing from the CLH report. The DS agreed and gave a short summary of the test in response to public consultation comments. The result of the test supported the conclusion that penflufen is not rapidly degradable. The MSCA also wanted to see temperatures mentioned in connection to the DT_{50} values. The DS explained that the basis of the presented DT_{50}s is included in the text in section 5.1.2.3. For the simulation study, DT_{50}s are based on study temperature. These were not adjusted to an environmentally relevant temperature, on the basis that they are high values and such an adjustment would not impact the classification. The MSCA

also wanted to add a fish BCF value used in the pesticide risk assessment (DAR, Volume 3, B.9 August 2011). However, the DS felt that the approach used to derive the BCF is not consistent with the assessment of bioaccumulation for hazard classification.

Assessment and comparison with the classification criteria

Penflufen was stable to hydrolysis. There was no ready biodegradation study available but in the water/sediment test, minimal mineralisation was observed with a maximum of 3.2% AR after 120 days. The geometric mean DT_{50} for the whole system was 221 days. The degradants were less toxic than the parent substance. The classification of the degradants was not considered further in the CLH Report because it was not needed to assess rapid degradability of penflufen. Based on the low mineralisation and a DT_{50} greater than 16 days in the water/sediment test, penflufen is considered not rapidly degradable.

Penflufen has no potential to bioaccumulate. The fish steady state BCF was 12 L/kg wet weight. Whole fish kinetic BCFs were between 100 and 103 L/kg. The log K_{OW} value was 3.3 at pH 4, pH 7 and pH 10. The whole fish BCF for penflufen is below the CLP trigger of ≥ 500 intended to identify substances with a potential to bioconcentrate. In addition the log K_{OW} is below the CLP trigger value of ≥ 4.

There were acute data available on fish, invertebrates, algae and Lemna*.* The lowest acute aquatic toxicity value is a 96h LC_{50} of 0.103 mg/L for *Cyprinus carpio*. The value of 0.103 mg/L fulfils the criteria for Aquatic Acute 1, *i.e.* < 1 mg/L. The value is in the range of 0.1 < $L(E)C_{50}$ ≤ 0.01, thus giving an acute M-factor of 1.

There were chronic data available on fish, invertebrates, algae and Lemna. The lowest value was a 35d NOEC of 0.0234 mg/L for *Pimephales promelas*. The value of 0.0234 mg/L fulfils the criteria for Aquatic Chronic 1, *i.e.* ≤ 0.1 mg/L for a non-rapidly degradable substance. The value is in the range 0.01 < NOEC ≤ 0.1, thus giving a chronic M-factor of 1.

Overall, RAC agrees with the DS proposal to **classify penflufen as Aquatic Acute 1 and Aquatic Chronic 1 with an M-factor of 1 for both acute and chronic classifications.**

Additional references

Prinsen, *et al*. (1997) Skin sensitization testing: the relevance of rechallenge and pretreatment with sodium lauryl sulfate in the guinea pig maximization test. Food and Chemical Toxicology 35:923-926

Hall, *et al*. (2012) Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes–conclusions from the 3rd International ESTP Expert Workshop. Toxicologic Pathology 40:971-994

Peffer, *et al*. (2018) Minimum datasets to establish a CAR-mediated mode of action for rodent liver tumours. Regulatory Toxicology and Pharmacology 96:106-120

Alison, *et al*. (1994) Neoplastic lesions of questionable significance to humans. Toxicologic Pathology 22:179-186

Greaves, (2012) Histopathology of preclinical toxicity studies: interpretation and relevance in drug safety evaluation. 4th ed. Academic Press, London.

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).