Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

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

Adopted

15 October 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Penflufen

EC Number: **Not assigned**

CAS Number: **494793-67-8**

Index Number: **Not assigned**

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Version number: **2** **Date:** **July 2017**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 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

CONTENTS

Part A.

Part B.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Penflufen***
EC number:	***Not allocated***
CAS number:	***494793-67-8***
Annex VI Index number:	***Not yet assigned***
Degree of purity:	***≥ 98%***
Impurities:	***There are a number of process impurities, these have been taken into account but are not considered to impact on the proposed classification. Please refer to the IUCLID for full details.***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Not currently listed
Current proposal for consideration by RAC	Carc 2; H351 – Suspected of causing cancer Aquatic Acute 1; H400: Very toxic to aquatic life, Acute M factor = 1 Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects, Chronic M factor = 1
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Carc 2; H351 – Suspected of causing cancer Aquatic Acute 1; H400: Very toxic to aquatic life, Acute M factor = 1 Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects, Chronic M factor = 1

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 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

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

2.15.	Organic peroxides	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	**Carc 2; H351 – Suspected of causing cancer**	Not applicable	Not classified	-
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1; H400** **Aquatic Chronic 1; H410**	**Acute M factor = 1** **Chronic M factor = 1**	Not classified	-
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors

[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictogram(s):	GHS08, GHS09
Signal word:	Danger
Hazard statements:	H351: Suspected of causing cancer
	H410: Very toxic to aquatic life with long lasting effects,
Precautionary statements:	Precautionary statements not included in Annex VI

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Penflufen is a fungicidal active substance that was approved for use as a plant protection product under Directive 91/414/EEC with the UK as the Rapporteur Member State (Regulation EU 1031/2013). In addition penflufen is being evaluated as a new biocidal active substance, for use as a wood preservative, in scope of Regulation (EU) 528/2012. Penflufen is also a new biocidal active substance for use as a wood preservative, in scope of Regulation (EU) 528/2012. The substance is not listed on Annex VI of CLP and has not previously been reviewed for harmonised classification and labelling in the EU.

At the time of the submission the substance is not registered under REACH.

2.2 Short summary of the scientific justification for the CLH proposal

Penflufen does not meet the criteria for classification for physical hazards.

The acute oral and dermal LD_{50} values were above those relevant for classification. Via the inhalation route, the 4hr LC_{50} was reported to be > 2.02 mg/L with 2.02 mg/l being the maximum achievable concentration. Therefore, the criteria for classification for acute toxicity via the inhalation route are not met. Signs of toxicity observed after single exposure were transient and did not lead to any significant functional changes. Further, there were no signs of respiratory tract irritation or narcotic effects. As such, the criteria for classification for STOT-SE are not met. No signs of skin irritation were observed and only minimal and reversible signs of eye irritation (conjunctival redness and chemosis with scores below those relevant for classification) were observed. As such the criteria for classification are not met. In a Guinea Pig maximisation test, positive responses were noted in 25% of tested animals. This is below the 30% considered for a positive result and, as such, the criteria for classification are not met. There is no data to inform on respiratory sensitisation.

Following repeated-exposure, the most sensitive target organ was found to be the liver. Changes in the liver were seen in all species tested (rats, mice and dogs) but in most cases these findings occurred at doses that were higher than the relevant guidance value for classification for STOT RE; the exception being the 28-day rat and 28-day dog studies. It is concluded that the liver effects at doses below the guidance values for classification were minimal and there was no consistent or conclusive evidence of hepatotoxicity. Exocrine single cell necrosis was reported in the pancreas in a 90 day rat study at doses relevant for classification. The same finding was noted in male rats only in the 1 year study, but there was no evidence of damage to the pancreas in the 2 year rat study at comparable doses. Further, no findings in the pancreas were reported in the mouse or dog studies. Overall, it is concluded that the effects seen in the rat studies were likely to be incidental and do not indicate a severe or significant toxic effect in the pancreas. Overall, it is considered that the criteria for classification for STOT-RE are not met.

The available data indicate that penflufen is not mutagenic *in vitro* or *in vivo* and therefore the criteria for classification are not met.

There were small increases in the incidence of hepatocellular adenoma in males and female rats. There was also an increased incidence of liver carcinoma in male mice treated with penflufen in the top and mid dose groups that exceeded the concurrent and historical control incidence rates. In addition, very small increased incidences of tumours in the ovary, haematopoietic system and brain

were observed in rats administered penflufen. The increased tumour frequencies were slight, only just outside control ranges and they could have arisen by chance. The increased frequencies of non-hepatic tumours were only evident in rats and some of the increases were of benign tumours only. A clear mechanistic basis for penflufen carcinogenicity is lacking (the possibility that a mode of action involving CAR activation was responsible for the slight increases in liver cancer has not been established unequivocally). If penflufen did produce a biologically significant tumour response in rats and mice, this was very weak. A case could be made for no classification, on the basis of a lack of relevance to humans. However, as discussed in detail in this proposal, relevance to humans cannot be dismissed for all the tumour types and the small increases above background levels make it difficult to conclude that they were incidental. Under these circumstances, the data appear to match the criteria for a Category 2 classification. Therefore it is proposed to classify penflufen with **Carc 2; H351 – Suspected of causing cancer**. This is in line with the EFSA conclusion (EFSA Journal 2012;10(8):2860), which raised concern for classification with Carc 2; based on the presence of these tumours.

There was no evidence that penflufen had a specific effect on fertility, sexual function or reproduction. Penflufen did not result in any adverse effects on developmental toxicity in the rat. In rabbits, an increase in dead fetuses in the high-dose group occurred together with maternal toxicity. In conclusion, there was no evidence that penflufen had a specific effect on development. Overall the criteria for classification are not met and it is not proposed to classify for reproductive toxicity.

For the purpose of classification, penflufen is considered not rapidly degradable and is not considered to have potential to bioaccumulate.

Aquatic acute toxicity data on penflufen are available for fish, invertebrates, algae and aquatic plants. Fish are the most acutely sensitive trophic group with Common Carp (*Cyprinus carpio*) marginally the most sensitive followed by Fathead Minnow (*Pimephales promelas*). The lowest acute value is a 96-hour LC_{50} is 0.103 mg a.s./l. On this basis penflufen should be classified as **Aquatic Acute 1; H400 – Very toxic to aquatic life, with an M factor of 1.**

Adequate chronic toxicity data on penflufen are available for fish, invertebrates, algae and aquatic plants. The lowest value is a 35-day NOEC for Fathead Minnow (*Pimephales promelas*) of 0.0234 mg a.s./l. Given this is 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; H410 – Very toxic to aquatic life with long lasting effects, with an M factor of 1**.

This is in line with the environmental classification in the EFSA conclusion.

2.3 Current harmonised classification and labelling

Not currently listed on Annex VI.

2.4 Current self-classification and labelling

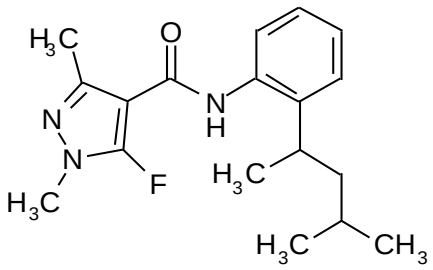
2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The following entries were provided in the Classification and Labelling Inventory at the time of submission.

Classification		Labelling		Number
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Pictograms, Signal Word Code(s)**	
Aquatic Acute 1 Aquatic Chronic 1	H400 H410	H400 H410	GHS09 Wng	30
Aquatic Acute 1 Aquatic Chronic 1	H400 H410	H410	GHS09 Wng	23
Aquatic Acute 1 Aquatic Chronic 1	H400 H410	H410	GHS09 Wng	1

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.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Penflufen is a fungicidal active substance. In 2013 under Regulation EU 1031/2013, a positive opinion was given to approve penflufen as a plant protection product under Council Directive 91/414/EEC with the UK as the Rapporteur Member State. It is also in the process of being evaluated for use in the EU as a fungicidal seed treatment on wheat and barley.

Penflufen is also a new biocidal active substance for use as a wood preservative, in scope of Regulation (EU) 528/2012.

In accordance with Article 36(2) of Regulation (EC) 1272/2008 on classification, labelling and packaging of substances and mixtures, penflufen should be considered for harmonised classification and labelling. As there is no existing entry in Annex VI of CLP, all hazard classes are considered in this proposal.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

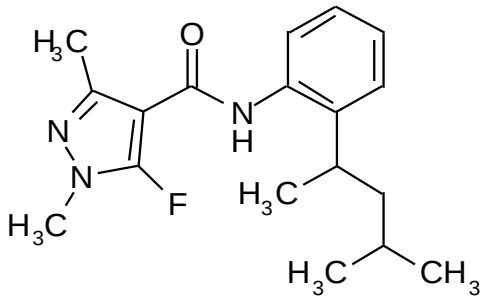
1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	Not allocated
EC name:	Not allocated
CAS number (EC inventory):	Not listed
CAS number:	494793-67-8
CAS name:	1H-Pyrazole-4-carboxamide, N-[2-(1,3-dimethylbuyl)phenyl]-5-fluoro-1,3-dimethyl-
IUPAC name:	2'-[(*RS*)-1,3-dimethylbutyl]-5-fluoro-1,3- dimethylpyrazole-4-carboxanilide*
CLP Annex VI Index number:	Not applicable
Molecular formula:	$C_{18}H_{24}FN_3O$
Molecular weight range:	317.41 g/mol

***** As included in the EFSA conclusion.

Structural formula of penflufen:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Penflufen (Racemic mixture)	≥ 98%		In the DAR and the approval notice the minimum purity was > 95% but in full-scale production the purity of the specification has increased to > 98%.

Current Annex VI entry: Not listed.

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
All impurities are confidential	Process impurities are individually present at < 2%		

There are a number of impurities in the technical material. These have been taken into consideration and are not considered to impact on the classification proposed in this dossier. Further information on the impurities is considered to be confidential but further details are provided in the technical dossier.

Current Annex VI entry: A number of impurities have a harmonised classification in Annex VI of CLP (refer to the IUCLID for full information). However, given the concentration at which they are present and the data available on penflufen, they are not considered to individually contribute to the classification.

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry: Not applicable.

1.2.1 Composition of test material

The material used in the available studies is considered to be equivalent to the material outlined above.

1.3 Physico-chemical properties

The physiochemical properties of penflufen are summarised below. Further reference can be found in the Draft Assessment Report (DAR) – Volume 3, Annex B.2: Physical and chemical properties – August 2011 and the Draft Competent Authority Report (dCAR) 2016. All studies were conducted to GLP and are considered to be adequate and reliable.

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Off white powder	Unpublished Study (ref). 2007 DAR B2.1.7/B2.1.8	Observation Purity 99.2%
Melting/freezing point	111.1°C	Unpublished Study (ref). 2007 DAR B.2.1.1	EEC Method A1 Purity 99.2%
Boiling point	Decomposes from 320°C	Unpublished Study (ref). 2007 DAR B.2.1.2	EEC Method A2 Purity 99.2%
Relative density	1.21 at 20 °C	Unpublished Study (ref). 2008 DAR B.2.1.4	EEC Method A3 Purity 99.2%
Vapour pressure	4.1 x 10-7 Pa at 20 °C 1.2 x 10-6 Pa at 25 °C 1.7 x 10-4 Pa at 50 °C Extrapolated	Unpublished Study (ref). 2007 DAR B.2.1.5	EEC Method A4 Purity 99.2%
Surface tension	61.6 mN/m at 20 °C	Unpublished Study (ref). 2009 DAR B.2.1.24	EEC Method A5 Purity 98.1%
Water solubility	11 mg/L at pH 4 10.09 mg/L at pH7 11.2 mg/L at pH9 All at 20 °C	Unpublished Study (ref). 2009 DAR B.2.1.11	EEC Method A6 Purity 99.2%
Partition coefficient n-octanol/water	Log Pow at 25 °C 3.3 at pH4, 7 and 9	Unpublished Study (ref). DAR B.2.1.13.	EEC Method A8 Purity 99.2%
Flash point	Not applicable – melting point is 111.1 °C	-	-
Flammability	The test item could not be ignited, but melted.	Unpublished Study (ref). 2009 DAR B.2.1.20	EEC Method A10 Purity 98.1%
Explosive properties	DSC measurements showed an exothermal decomposition in the temperature range 270-410oC with an energy of 240 – 330 J/g.	Unpublished Study (ref). 2009 DAR B.2.1.22	EEC Method A14 Purity 98.1%

Self-ignition temperature	No self-ignition was observed up to the maximum test temperature of 403 ºC	Unpublished Study (ref). 2009 DAR B.2.1.20	EEC Method A16 Purity 98.1%
Oxidising properties	Maximum burning rate of the test item/cellulose mixture was 1.27 mm/s (40 and 50% penflufen). The maximum burning rate of the reference material (barium nitrate/cellulose mixture) was 1.32 mm/s. Mixtures of test item/Kieselghur were found to propagate combustion. No reaction with barium nitration/Kieselghur. Under an inert atmosphere mixtures of test item/cellulose (45 and 50% penflufen), did not ignite. Mixtures of barium nitrate/cellulose were found to ignite with a burning rate of 0.55 mm/s	Unpublished Study (ref). 2009 DAR B.2.1.23	EEC Method A17 Purity 98.1%
Granulometry	No data	-	-
Stability in organic solvents and identity of relevant degradation products	No data Solubility: Methanol: 126 g/L n-heptane: 1.6 g/L Toluene: 62 g/L Dichloromethane: >250 g/L Acetone: 139 g/L Ethyl acetate: 96 g/L Dimethyl sulfoxide: 162 g/L	- Unpublished Study (ref). 2009 DAR B.2.1.12.	- EEC A6 Purity 99.2%
Dissociation constant	No dissociation constant was found in aqueous solution. The molecule has no moieties prone to dissociation.	Unpublished Study (ref). 2009 DAR B.2.1.18	OECD 112 Purity 99.2%
Viscosity	Not relevant	-	-

2 MANUFACTURE AND USES

2.1 Manufacture

Penflufen is manufactured in the EU.

2.2 Identified use

Penflufen has been approved for use in the EU as a fungicidal seed treatment on potatoes and is in the process of being evaluated for use in the EU as a fungicidal seed treatment on wheat and barley.

Penflufen is also in the process of being evaluated under Regulation (EU) 528/2012 for use as a biocide in PT 8 in the EU.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 8			

3.1.1 Summary and discussion of physico-chemical properties

DSC measurements showed an exothermal decomposition in the temperature range 270-410^{o}C with an energy of 240 – 330 J/g.

In a flammability study in accordance with EEC A10, penflufen did not ignite but melted. Experience with handling and use indicates that the substance is not pyrophoric and does not emit flammable gases in contact with water.

In a standard study (EEC A.17), the maximum burning rate of the test item/cellulose mixtures was 1.27 mm/s (with 40 and 50% penflufen). The maximum burning rate of the reference material (barium nitrate/cellulose mixture) was 1.32 mm/s. Given that the burning rate with penflufen was similar to that with the reference material, additional studies were conducted with an inert material (Kieselghur) and under an inert atmosphere. Mixtures of test item/Kieselghur were found to propagate combustion whereas no reaction was noted with the reference material (barium nitrate)/Kieselghur mixture. Under an inert atmosphere, mixtures of test item/cellulose (45 and 50% penflufen) did not ignite. Mixtures of barium nitrate/cellulose were found to ignite with a burning rate of 0.55 mm/s.

3.1.2 Comparison with criteria

A substance is considered for classification as an explosive substance where a positive result is obtained in the test series indicated in figure 2.1.2 of Annex I of the CLP regulation. In a preliminary DSC screen, an exothermal decomposition in the temperature range 270-410^{o}C with an energy of 240 – 330 J/g was observed. As the decomposition energy was less than 500 J/g and the onset of decomposition was below 500 ^{o}C a full study was not required and the substance does not meet the criteria for classification as explosive in accordance with section 2.1.4.3(c) of Annex I of CLP.

A substance (non-metal) is classified as a flammable solid when the burning time is < 45 seconds or the burning rate is > 2.2 mm/s. Penflufen melted but did not ignite on exposure to a flame and therefore, the criteria for classification as a flammable solid are not met.

Experience in handling and use indicates that penflufen is not pyrophoric and does not emit flammable gases on contact with water. Therefore, the criteria for classification in these hazard classes are not met.

A substance is classified as an oxidising solid when the burning time of a sample-to-cellulose mixture is less than or equal to the burning time of the appropriate reference sample. In an initial study the maximum burning rate of the test item/cellulose mixture was 1.27 mm/s, obtained with a 40% and 50% test item/cellulose mixture. This was comparable to the maximum burning rate (1.32

mm/s) obtained with the 55% barium nitrate/cellulose reference material. A further test with an inert material (Kieselguhr) was conducted. In this, test item/Kieselguhr mixtures were found to propagate combustion whereas the barium nitrate reference material did not. Under an inert atmosphere mixtures of penflufen/cellulose did not ignite whereas the reference material did with a burning rate of 0.5 mm/s. Considering the chemical structure of penflufen (which does contain oxygen and fluorine but only bound to carbon atoms) and the results of the available study, penflufen does not meet the criteria for classification as an oxidising solid.

3.1.3 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

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**.

4 HUMAN HEALTH HAZARD ASSESSMENT

Presented below is the key information pertinent to determining a classification position based, primarily, on the UK's review of penflufen in the pesticide Draft Assessment Report (DAR) made under Directive 91/414/EEC. This is also comparable with the assessment of the substance under Regulation (EC) 528/2012, as presented in the Draft Competent Authority Report (dCAR) 2016.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The toxicokinetics of penflufen have been investigated in rats after single high and low dose administration of radiolabelled penflufen. Toxicokinetics following a single high dose of 200 mg/kg bw had a similar profile to the low dose, with the exception that excretion was slower. No data are available following repeated exposure to penflufen. Other studies (see sections 4.7 and 4.9.3) show that enzyme induction occurs following repeated dosing of penflufen, but without repeat dose metabolism studies it is not known how enzyme induction will affect distribution and metabolism.

Absorption

In rats administered a single oral dose of 2 mg/kg bw radiolabelled penflufen, absorption was rapid ($t_{max} \leq 1.5$ hours) and extensive (approximately 93% in bile-cannulated male rats based on levels in carcass, urine and bile after 48 hours).

Distribution

Penflufen was widely distributed with highest concentrations occurring in the liver, kidneys and adrenals of both sexes, and the brown fat and Harderian gland in females.

Metabolism

Penflufen was extensively metabolised in the rat with less than 2% of administered dose excreted as parent compound. A large number of metabolites were detected, accounting for 60 to 95% of administered dose, but all were at levels below 10% (with the exception of a ketone present at levels up to ~ 17% in females). The pattern of metabolites formed was similar at 2 and 200 mg/kg bw and broadly similar in both sexes. Most of the metabolites were demethylated products of the pyrazole ring. Hydroxylation was another major metabolic reaction leading to trihydroxy and dihydroxy compounds.

Excretion

48 hours after administration of 2 mg/kg bw radiolabelled penflufen to male rats, urinary excretion and biliary excretion accounted for 21% and 70% of administered dose, respectively. Rapid excretion in the bile suggests there may be a significant oral 'first-pass' effect in the liver.

A difference between the sexes was noted in the pattern of excretion, with higher urinary excretion occurring in females (up to 59% of dose in females compared with 33% in males 168 hours after a dose of 5 mg/kg bw).

Excretion was rapid with approximately 80% excreted within 24 hours, and was essentially complete by 168 hours post dosing. Plasma concentrations declined to ≤1% of the maximum concentrations within 72 hours and the plasma elimination half-life was less than 24 hours. There was no evidence of accumulation.

The toxicokinetic profile of penflufen after inhalation and dermal exposure has not been investigated.

4.1.2 Human information

None available.

4.1.3 Summary and discussion on toxicokinetics

The toxicokinetics of penflufen was investigated in single oral dose studies in rats. Penflufen was rapidly and extensively absorbed and distributed. The high levels of biliary excretion provided evidence of 'first-pass' metabolism in the liver. The large number of metabolites identified indicated that penflufen is extensively metabolised.

References: DAR B.6.1 (unpublished studies).

Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

<table>
<tr><th colspan="3">Acute Oral</th></tr>
<tr><th>Method</th><th>LD_{50}</th><th>Observations and remarks</th></tr>
<tr><td>Rat: Wistar (6 females)

2000 mg/kg bw
Purity: 95.6%

Vehicle: tap water with 2% Cremophor EL

OECD 423
GLP

DAR B.6.2.1
Unpublished Study (ref). (2007a)</td><td>>2000 mg/kg bw</td><td>There were no mortalities, no clinical signs of toxicity and no adverse effects on body weight. No abnormalities were observed at necropsy.</td></tr>
<tr><td>Rat acute neurotoxicity: Crl:WI (Han)

Initial study: 12/sex/dose 0/100/500/2000 mg/kg bw

Follow up study: females only 12/dose 0/25/50 mg/kg bw

Purity 95.6%

Vehicle: 0.5% methylcellulose/0.4% Tween 80

OECD 424
GLP

DAR B.6.7
Unpublished Study (ref). (2009)</td><td>>2000 mg/kg bw</td><td><u>Initial study:</u>
There were no mortalities. Transient clinical signs are shown in the table below. There were no effects on body weight and no gross pathological findings at necropsy.

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

In both sexes there was a dose-related reduction in motor and locomotor activity in males at 500 and 2000 mg/kg bw, and in females at all dose levels on the day of dosing; these findings were reversible when measured on day 7.

Incidence of selected clinical signs, observed days 0-3
<table>
<tr><th rowspan="3">Observation</th><th colspan="8">Dose of Penflufen (mg/kg)</th></tr>
<tr><th colspan="4">Males</th><th colspan="4">Females</th></tr>
<tr><th>0</th><th>100</th><th>500</th><th>2000</th><th>0</th><th>100</th><th>500</th><th>2000</th></tr>
<tr><td>Urine staining</td><td>1</td><td>0</td><td>4</td><td>9</td><td>1</td><td>1</td><td>10</td><td>11</td></tr>
<tr><td>Stiff-legged gait</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>4</td><td>5</td></tr>
<tr><td>Ataxia</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>4</td><td>5</td></tr>
<tr><td>Decreased activity</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>4</td><td>5</td></tr>
</table>
<u>Follow-up study:</u> No mortalities and no treatment related findings.</td></tr>
</table>

Acute Inhalation		
Method	**LC50**	**Observations and remarks**
Rat: Wistar (5/sex/dose) Nose only exposure for 4 hours 2.02 mg/L (dust aerosol) MMAD approx. 4.11 µm, geometric standard deviation 1.67 Purity 95.6% OECD 403 GLP DAR 6.2.3 Unpublished Study (ref). (2007), amended (2008)	>2.02 mg/L (the highest technically achievable concentration).	There were no mortalities. Clinical signs in 4 out of 5 animals of each sex persisted for up to 3 days after exposure and consisted of bradypnoea, laboured breathing patterns, reduced motility, piloerection, red incrustations on the nose, gait: high legged, staggering. Rectal temperature after exposure was significantly lower compared with recent control group (35.0 vs. 38.0 ˚C in males and 34.4 vs. 38.0˚C in females). No abnormalities were observed in the lungs at gross pathological examination.
Acute Dermal		
Method	**LD50**	**Observations and remarks**
Rat: Wistar, (5/sex/dose) 2000 mg/kg bw moistened with tap water Purity 95.6% OECD 402 GLP DAR 6.2.2 Unpublished Study (ref). (2007b)	>2000 mg/kg bw	There were no mortalities, no clinical signs of systemic toxicity, no local signs of irritation and no adverse effects on body weight. No abnormalities were observed at necropsy.

4.1.4 Non-human information

4.1.4.1 Acute toxicity: oral

Two GLP and guideline-compliant reliable studies are available. In an acute oral study the oral LD_{50} of penflufen was > 2000 mg/kg bw in female rats (males not investigated). In an acute oral neurotoxicity study the LD_{50} of penflufen was > 2000 mg/kg bw in both sexes.

4.1.4.2 Acute toxicity: inhalation

In a GLP and guideline compliant reliable study the oral LC_{50} of penflufen in male and female rats after 4 hours exposure was > 2.02 mg/L (the highest achievable concentration).

4.1.4.3 Acute toxicity: dermal

In a GLP and guideline-compliant reliable study the dermal LD_{50} of penflufen was > 2000 mg/kg bw in male and female rats.

4.1.4.4 Acute toxicity: other routes

No data available.

4.1.5 Human information

No data available.

4.1.6 Summary and discussion of acute toxicity

Refer to section 4.2.1

4.1.7 Comparison with criteria

Via the oral route the LD_{50} can be identified as > 2000 mg/kg bw which is above the value for classification (≤ 2000 mg/kg bw). No classification is required.

Via the inhalation route the LC_{50} after 4 hours exposure can be identified as > 2.02 mg/L following exposure to penflufen as a dust aerosol. The value for classification of a dust/mist is ≤ 5mg/L however, since 2.02 mg/L was the maximum achievable concentration no classification is proposed.

Via the dermal route the LD_{50} can be identified as > 2000 mg/kg bw which is above the value for classification (≤ 2000 mg/kg bw). No classification is required.

4.1.8 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification.

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 Year: 2009	OECD TG 424 GLP 12/sex/dose Doses: 0, 100, 500, 2000 mg/kg bw	> 2000 mg/kg bw	No mortalities Transient clinical signs (resolved by day 3): stiff-legged gait, ataxia, 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.

4.2 Specific target organ toxicity – single exposure (STOT SE)

4.2.1 Summary and discussion of Specific target organ toxicity – single exposure

A summary of the effects observed following single exposure in animal studies is provided in table 10. Also refer to section 4.4.3 for information on respiratory irritation.

There was no evidence of any irreversible or delayed effects following single exposure to penflufen. There were no clinical signs of toxicity in the acute oral study in rats at a dose of 2000 mg/kg bw. Some reversible signs of toxicity were evident in rats in an acute oral neurotoxicity study, however these signs are concluded to be attributable to general acute toxicity. Clinical signs of toxicity were also seen in rats following a four hour inhalation exposure to penflufen as an aerosol dust at the maximum attainable concentration of 2.02mg/L. These signs are probably attributable to general toxicity and exposure to a dust.

There were no indications of neurotoxicity in a 13 week subchronic neurotoxicity study in rats at doses up to 600 mg/kg bw (neurotoxicity is discussed in section 4.11.1.1).

Pathological examination did not reveal any severe target organ effects in any of the studies at necropsy.

4.2.2 Comparison with criteria

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance.

Classification as STOT-SE1 and STOT-SE 2 is based on evidence associating a single exposure with a consistent and significant toxic effect that could indicate significant functional changes that are more than transient in nature, such as significant organ damage observed at necropsy.

Signs of toxicity were evident following single exposure to penflufen via the oral and inhalation routes in rats, but these were transient and did not lead to any significant functional changes in any organs. Therefore it is concluded that classification as STOT-SE1 or STOT-SE 2 is not justified.

Classification as STOT-SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation. According to the CLP classification criteria clinical signs in animals that may indicate narcotic effects may include lethargy, lack of coordination, loss of righting reflex, and ataxia.

There were no conclusive signs of respiratory tract irritation (see section 4.4.3.3) or narcotic effects, therefore it is concluded that classification as STOT-SE 3 is not justified.

4.2.3 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification.

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.

4.3 Irritation

4.3.1 Skin irritation

Penflufen's potential to cause skin irritation has been investigated in the rabbit.

Table 11: Summary table of relevant skin irritation studies

Method	Results	Remarks
OECD 404 GLP New Zealand White Rabbits 3 females 4 hours exposure to penflufen, purity 95.6%, moistened with water. DAR 6.2.4 Unpublished Study (ref). (2007a)	Average score for each animal (mean of 24, 48, 72 h observations) Erythema: 0, 0, 0 Oedema: 0, 0, 0	Not a skin irritant

4.3.1.1 Non-human information

The skin irritation potential of penflufen has been investigated in one GLP and guideline compliant study conducted in rabbits. No signs of irritation were observed.

4.3.1.2 Human information

No data available.

4.3.1.3 Comparison with criteria

Classification is required where the mean score for erythema or oedema is ≥ 2.3 or ≥ 2 respectively in 2 out of 3 animals (average from observations at 24, 48 and 72 hours) or where effects persist until the end of the observation period. As the mean scores were all O, penflufen does not meet the criteria for classification as a skin irritant.

4.3.1.4 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification.

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.

4.3.2 Eye irritation

Penflufen's potential to cause eye irritation has been investigated in the rabbit.

Table 12: Summary table of relevant eye irritation studies

Method	Results	Remarks
OECD 405 GLP New Zealand White Rabbits 3 females Pulverised penflufen, purity 95.6% DAR 6.2.5 Unpublished Study (ref). (2007b)	Average score for each animal (mean of 24, 48, 72 h observations) Cornea opacity: 0, 0, 0 Iris lesion: 0, 0, 0 Conjunctiva redness: 0.7, 0.7, 0.7 Conjunctiva chemosis: 0.3, 0, 0	All signs of irritation were reversible within 72 h. Not an eye irritant.

4.3.2.1 Non-human information

The eye irritation potential of penflufen has been investigated in a standard guideline-compliant GLP study in rabbits. No effects on the cornea or iris were noted. Redness and chemosis were observed in the conjunctiva in all rabbits but these were fully reversible within 72 hours.

4.3.2.2 Human information

No data available.

4.3.2.3 Comparison with criteria

Mild signs of eye irritation were observed in a guideline-compliant study conducted in rabbits. The mean scores at 24, 48 and 72 hours for each animal using grading according to Draize were 0.7 for conjunctival redness, and a maximum of 0.3 for chemosis. These are below the minimum scores for classification, which are ≥ 2 for conjunctival redness, and ≥ 2 for chemosis (mean individual animal score from observations at 24, 48 and 72 hours). Further, no effects on the cornea or iris were observed. In conclusion penflufen does not require classification as an eye irritant.

4.3.2.4 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

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.

4.3.3 Respiratory tract irritation

4.3.3.1 Non-human information

The respiratory tract irritation potential of penflufen has not been investigated directly in animals. Penflufen is not a skin or eye irritant. In an acute inhalation toxicity study (section 4.2), clinical signs seen after exposure to 2.02 mg/L penflufen were bradypnoea, laboured breathing patterns, and red incrustations on the nose. These signs 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 tract irritation. Gross pathological examination at necropsy did not reveal any adverse findings in the lungs that would be indicative of an irritant effect. There are no repeat dose inhalation exposure studies conducted on penflufen.

4.3.3.2 Human information

No data available.

4.3.3.3 Comparison with criteria

There is no evidence to indicate that penflufen is a respiratory tract irritant. It is therefore concluded that classification is not required.

4.3.3.4 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification.

4.4 Corrosivity

4.4.1 Non-human information

See section 4.4.1.

4.4.2 Human information

No information available.

4.4.3 Summary and discussion of corrosivity

Penflufen did not lead to any signs of corrosion or skin damage in a well-conducted GLP and guideline-compliant skin irritation study conducted in the rabbit (see section 4.4.1). No human data are available.

4.4.4 Comparison with criteria

No signs of corrosivity were observed in a skin irritation study conducted in the rabbit. Penflufen does not require classification as corrosive.

4.4.5 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification.

4.5 Sensitisation

4.5.1 Skin sensitisation

One skin sensitisation study has been conducted on Penflufen.

Table 13: Summary table of relevant skin sensitisation studies

Species/Method	Doses	No. sensitised/total no.	Result

<table>
<tr>
<td>Magnusson and Kligman maximisation test

Guinea-pig,
10 controls,
20 treated.

Purity 95.6%

OECD 406
GLP

DAR 6.2.6
Unpublished Study (ref).
(2007)</td>
<td>Induction:
Intradermal: 2.5% suspension in polyethylene glycol 400

Topical: 50% suspension in polyethylene glycol 400

Challenge: 50% suspension in polyethylene glycol 400</td>
<td>
<table>
<tr><td></td><td>Control</td><td>Test</td></tr>
<tr><td colspan="3">1st challenge 50%</td></tr>
<tr><td>48h</td><td>0/10</td><td>5/20</td></tr>
<tr><td>72h</td><td>0/10</td><td>4/20</td></tr>
<tr><td colspan="3">2nd challenge 50%</td></tr>
<tr><td>48h</td><td>0/10</td><td>2/20</td></tr>
<tr><td>72h</td><td>0/10</td><td>0/20</td></tr>
</table>
Positive control using alpha hexyl cinnamic aldehyde in polyethylene glycol 400 confirmed the reliability of the test.
</td>
<td>Negative.
A deficiency in the study was that the dermal induction dose did not cause any skin irritation. According to OECD 406 a solution of 10% sodium lauryl sulphate in Vaseline should have been applied to create local irritation 24h prior to the dermal induction dose.</td>
</tr>
</table>

4.5.1.1 Non-human information

Skin sensitisation was investigated in a Magnusson and Kligman maximisation test (an adjuvant-type test) in the guinea-pig. The test was conducted to GLP and followed OECD 406 test guidelines with the exception that the dermal induction dose did not cause any skin irritation (see table above).

At a challenge dose of 50% there was a 25% positive response at first challenge, and a 10% positive response at rechallenge. Signs of irritation were seen following intradermal induction.

4.5.1.2 Human information

No information available.

4.5.1.3 Comparison with criteria

In an adjuvant-type skin sensitisation study conducted in guinea-pigs a positive skin reaction was seen in 25% of animals after first challenge (10% positive response at re-challenge). A response of 30% is considered as positive in such a study and therefore it is concluded that penflufen does not meet the criteria for classification for skin sensitisation based on the results of this study.

4.5.1.4 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification.

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.

Additional key elements

Upon request, the full study report from the skin sensitisation study has been made available to RAC. The individual animal data from the treated group are presented in the following table.

Individual animal data for the treated group in the guinea pig maximization test[a]				
Animal no.	**1st challenge**		**2nd challenge**	
	48 h	**72 h**	**48 h**	**72 h**
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	1	1	1	0
17	0	0	0	0
18	1	1	0	0
19	0	0	0	0

20	0	0	0	0
21	1	0	0	0
22	1	1	1	0
23	1	1	0	0
24	0	0	0	0
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0

[a] Severity scores: 0 = no reaction; 1 = slight localized redness; 2 = moderate confluent redness; 3 = severe redness and swelling

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 rowspan="2"></td><td colspan="2">No. sensitised/total no.</td></tr>
<tr><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**.

4.5.2 Respiratory sensitisation

4.5.2.1 Non-human information

No data are available.

4.5.2.2 Human information

No data are available.

4.5.2.3 Comparison with criteria

No data are available.

4.5.2.4 Conclusions on classification and labelling

Not classified - data lacking.

4.6 Repeated dose toxicity

The short term and repeated-dose toxicity of penflufen has been studied extensively in standard GLP/OECD-compliant studies involving repeated oral treatment of rats (28-day, 90-day), mice (28-day and 90-day) and dogs (28-day, 90-day and 1 year). Exposure via the dermal route has been investigated in rats in a 28-day study. In addition, there are chronic toxicity studies in rats (1 year, and 1 year with 3 month recovery) which were conducted as part of a two year carcinogenicity study. No repeated dose inhalation toxicity studies are available.

Table 14: Summary table of relevant repeated dose oral toxicity studies

Note: The LOAEL values are given for information only. They have been taken directly from documentation connected to the EFSA peer review of penflufen without further critical assessment.

Method	Dose Levels	Observations and Remarks (main toxicological effects)
28 day oral dietary Rat: Wistar 5/sex/dose Penflufen purity 99.2% or 99.4% Liver microsomes were analysed for cytochrome P-450 content and ethoxyresorufin-O-deethylase (EROD), pentoxyresorufin-O-dependentylase (PROD) and benzoxyresorufin-O-debenzylase (BROD) activity. Non-guideline GLP: No DAR 6.3.1 Unpublished Study (ref). (2004)	0, 150, 2000, 7000 ppm corresponding to 0, 12/13, 154/169, 560/648 mg/kg bw/day in m/f **STOT RE guidance value in rat 28 day study is ≤ 300 mg/kg bw/day**	There were no deaths or clinical signs at any dose. **150 ppm (12/13 mg/kg bw/day)** No adverse effects. **2000 ppm (154/169 mg/kg bw/day)** **Males:** Organ weights: ↑ relative liver weight (11%), Enzyme activity: ↑ cytochrome P450 (14%), ↑ BROD (330%) and ↑ PROD (140%) **Females:** ↓ bodyweight gain (16%), ↓ food consumption Clinical chemistry: 27% ↑ cholesterol Enzyme activity: ↑ cytochrome P450 (32%), ↑ BROD (774%) and ↑ PROD (372%) Histopathology: centrilobular hepatocyte hypertrophy (in 2/5 f versus 0/5 f in controls). **7000 ppm (560/648 mg/kg bw/day)** **Males:** Organ weights: ↑ absolute and relative liver weight (32/26%). Enzyme activity: ↑ cytochrome P450 (9%), ↑ BROD (547%) and ↑ PROD (92%) Histopathology: centrilobular hepatocyte hypertrophy (in 5/5 m versus 0/5 m in controls). **Females:** ↓ bodyweight gain (12%), ↓ food consumption Organ weights: ↑ absolute and relative liver weight (15/19%). Clinical chemistry: 31% ↑ cholesterol, 45% ↓ bilirubin Enzyme activity: ↑ cytochrome P450 (53%), ↑ BROD (2293%) and ↑ PROD (440%) Histopathology: centrilobular hepatocyte hypertrophy (in 5/5 f versus 0/5 f in controls) *LOAEL 2000 ppm (154/169 mg/kg bw/day)*

29/30 day oral dietary immunotoxicity Rat: Wistar 8/sex/dose Penflufen purity 95.6% Administered in diet for 29 days in males and 30 days in females. 5 days before scheduled kill sheep erythrocytes were administered intravenously for a plaque forming cell assay (PFCA). A positive control group treated with a known immuno-suppressant was not included, however the validity of the plaque-forming cell assay had been previously demonstrated with cyclo-phosphamide. Bodyweight and weights of spleen and thymus measured. No histopathology conducted. EPA OPPTS 870.7800 GLP: Yes DAR B.6.8.2 Unpublished Study (ref). (2008)	0, 200, 1000, 7000 ppm corresponding to 0, 18/20, 83/104, 756/960 mg/kg bw/day. **STOT RE guidance value for rat 28 day study is ≤ 300 mg/kg bw/day**	There were no deaths or clinical signs of toxicity, including immunotoxicity at any dose. **200 ppm (18/20 mg/kg bw/day)** No adverse effects. **1000 ppm (83/104 mg/kg bw/day)** No adverse effects. **7000 ppm (756/960 mg/kg bw/day)** **Males:** ↓ bodyweight gain (17%), ↑ food consumption (30%). **Females:** ↓ bodyweight gain (59%), ↑ food consumption (25%).

90 day oral dietary Rat: Wistar 10/sex/dose Penflufen purity 98.8% Included a neurotoxicity assessment of motor activity, sensory reactions, and grip strength at the end of the treatment period. OECD 408 GLP: Yes DAR 6.3.1 Unpublished Study (ref). (2006a)	0, 150, 7000, 14,000 ppm corresponding to 0, 9.5/11.4, 457/492, 949/1009 mg/kg bw/day in m/f **STOT RE guidance value in rat 90 day study is ≤ 100 mg/kg bw/day**	There were no deaths or clinical signs of toxicity at any dose. **150 ppm (9.5/11.4 mg/kg bw/day)** **Males:** Organ weights: ↑ absolute liver weight (11%) Histopathology: pancreas increase of exocrine single cell necrosis (in 5/10 m versus 0 in control m). **Females:** No adverse effects. **7000 ppm (457/492 mg/kg bw/day)** **Males:** Organ weights: ↑ absolute and relative liver weight (34/35%). Histopathology: Liver: centrilobular hepatocellular hypertrophy (in 10/10 m versus 0/10 m in controls). Thyroid: follicular cell hypertrophy (in 8/10 m versus 0 in controls) and focal/multifocal colloid alteration (in 3/10 m versus 0 in control m). Kidney: focal/multifocal tubular hyaline droplets (in 5/10 m versus 2/10 in control m) Pancreas: increase of exocrine single cell necrosis (in 4/10 m versus 0 in control m). Pituitary: basophil cell hypertrophy (6/10m compared to 3/10 m in control m) **Females:** ↓ bodyweight gain (17%) & ↓ food consumption. Organ weights: ↑ absolute and relative liver weight (18/26%) Clinical chemistry: 36% ↑ cholesterol, 35% ↓ bilirubin, 200% ↑ gamma-glutamyltransferase Histopathology: centrilobular hepatocellular hypertrophy (in 10/10 f versus 0/10 f in controls) **14,000 ppm (949/1009 mg/kg bw/day)** **Males:** Organ weights: ↑ absolute and relative liver weight (56/64%) Clinical chemistry: 58% ↑ cholesterol, 300% ↑ gamma-glutamyltransferase Histopathology: Liver: centrilobular hepatocellular hypertrophy (in 9/10 m versus 0/10 m in controls) Thyroid: follicular cell hypertrophy (in 8/10 m versus 0 in control m), focal/multifocal colloid alteration (in 3/10 m versus 0 in control m) Kidney: focal/multifocal tubular hyaline droplets (in 6/10 m versus 2/10 in control m). Pancreas: increase of exocrine single cell necrosis (in 4/10 m versus 0 in control m). Pituitary: basophil cell hypertrophy (5/10 m versus 3/10 in control m)

		Females: ↓ bodyweight gain (12%), ↓ food consumption Organ weights: ↑ absolute and relative liver weight (31/39%) Clinical chemistry: 27% ↑ cholesterol, 43% ↓ bilirubin, 300% ↑ gamma-glutamyltransferase, 43%↑ alanine aminotransferase Histopathology: Liver: centrilobular hepatocellular hypertrophy (in 10/10 f versus 010 f in controls) Pancreas: increase of exocrine single cell necrosis (4/10 f versus 0 in control f) Thyroid: follicular cell hypertrophy (6/10 f versus 0 in control f). *LOAEL 7000 ppm (457/492 mg/kg bw/day)*
90 day oral dietary Rat: Wistar 10/sex/dose Penflufen purity 98.8% In a deviation from the study guidelines only the kidney, liver, pancreas, pituitary and thyroid glands were examined microscopically. OECD 408 GLP: Yes DAR 6.3.1 Unpublished Study (ref). (2006b)	0, 50, 150, 3500 ppm corresponding to 0, 3.2/3.7, 9.3/11.4, 228/260 mg/kg bw/day in m/f **STOT RE guidance value in rat 90 day study is ≤ 100 mg/kg bw/day**	There were no deaths or clinical signs of toxicity at any dose. **50 ppm (3.2/3.7 mg/kg bw/day)** No adverse effects. **150 ppm (9.3/11.4 mg/kg bw/day)** No adverse effects. **3500 ppm (228/260 mg/kg bw/day)** **Males:** One male euthanized on day 69 for humane reasons displayed clinical signs and adverse findings at necropsy that were not considered to be treatment-related. Organ weights: ↑ absolute and relative liver weight (15/16%) Histopathology: Liver: centrilobular hepatocellular hypertrophy (in 2/9 m versus 0/9 m in controls) Kidney: focal/multifocal tubular hyaline droplets (in 3/9 m versus 0 in control m). **Females:** ↓ bodyweight gain, Organ weights: ↑ absolute and relative liver weight (9/16%) Histopathology: Liver: centrilobular hepatocellular hypertrophy (in 5/10 f versus 0/10 f in controls) *LOAEL 3500 ppm (228/260 mg/kg bw/day)*

90 day oral dietary neurotoxicity Rat: Wistar 12/sex/dose Penflufen purity 95.6% A functional observational battery and motor/ locomotor activity measurements were conducted on study weeks -1, 2, 4, 8 and 13. Histopathology was confined to examination of brain and nervous system tissue of the control and high dose group. OECD 424 GLP: Yes DAR B.6.7 Unpublished Study (ref). (2009)	0, 250, 2000, 8000 ppm corresponding to 0, 16.0/19.9, 126/156, 516/609 mg/kg bw/day. **STOT RE guidance value in rat 90 day study is ≤ 100 mg/kg bw/day**	There were no deaths or clinical signs of toxicity at any dose. **250 ppm (16.0/19.9 mg/kg bw/day)** No adverse effects. **2000 ppm (126/156 mg/kg bw/day)** **Males:** ↑ relative liver weight (13%) **Females:** ↓ food consumption, ↑relative liver weight (12%). **8000 ppm (516/609 mg/kg bw/day)** **Males:** ↓ bodyweight gain (11%), ↓ food consumption, ↑ absolute and relative liver weight (21/23%) **Females:** ↓ bodyweight gain (30%), ↓ food consumption, ↑ relative liver weight (28%). *LOAEL 2000 ppm (126/156 mg/kg bw/day)*

1 year dietary Rat: Wistar 10/sex/dose Penflufen purity 95.6% (study conducted as part of the 2 year carcinogenicity study) Microscopic examination on the liver, lung, kidney, and thyroid gland of all dose groups. For all other organs only control and high dose groups were examined microscopically, and any organs from other dose groups with gross abnormalities, and all organs in animals that died before the end of the study. OECD 453 GLP: Yes DAR B.6.5.1 Unpublished Study (ref). (2009)	0, 100, 2000, 7000 ppm corresponding to 0, 4.6/6.3, 90/126, 327/446 mg/kg bw/day. **STOT RE guidance value in rat 1 year study is ≤ 25 mg/kg bw/day calculated from the guidance value for a 90 day study.**	**100 ppm (4.6/6.3 mg/kg bw/day)** No adverse effects. **2000 ppm (90/126 mg/kg bw/day)** **Males:** Histopathology: hepatocellular macrovacuolation, mainly centrilobular diffuse (in 2/10 m versus 1/10 in control m). **Females:** ↓ bodyweight gain (16%), ↓ food consumption. Organ weights: ↑ relative liver weight (10%). Clinical chemistry: 11% ↑ cholesterol, 41% ↓ bilirubin. Histopathology: thyroid diffuse follicular cell hypertrophy (in 1/10 f versus 0 in control f). **7000 ppm (327/446 mg/kg bw/day)** **Males:** ↓ bodyweight gain (8%), ↓ food consumption. Organ weights: ↑ relative liver weight (25%). Clinical chemistry: 50% ↓ bilirubin Histopathology: Liver: centrilobular hepatocellular hypertrophy (in 10/10 m versus 0/10 m in controls), hepatocellular macrovacuolation, mainly centrilobular diffuse (in 7/10 m versus to 1/10 m in controls) Thyroid: diffuse follicular cell hypertrophy (in 3/10 m versus 0 in control m).

1 year dietary then 13 weeks recovery Rat: Wistar 10/sex/dose Penflufen purity 95.6% (study conducted as part of the 2 year carcinogenicity study) Microscopic examination was carried out on the liver in all dose groups, and the thyroid gland in the control, top and mid dose groups. For all other organs only the control and high dose groups were examined microscopically, and any organs from other dose groups with gross abnormalities, and all organs in any animals that died before the end of the study. OECD 453 GLP: Yes DAR B.6.5.1 Unpublished Study (ref). (2009)	0, 100, 2000, 7000 ppm corresponding to 0, 4.6/6.3, 90/126, 327/446 mg/kg bw/day for 1 year followed by 13 weeks recovery **STOT RE guidance value in rat 1 year study is ≤ 25 mg/kg bw/day, calculated from the guidance value for a 90 day study.**	There were no treatment-related effects on survival at any dose. **100 ppm (4.6/6.3 mg/kg bw/day)** No adverse effects. **2000 ppm (90/126 mg/kg bw/day)** No adverse effects. **7000 ppm (327/446 mg/kg bw/day)** **Males:** Clinical chemistry: bilirubin, 29% ↓ Organ weights: 19% ↑ relative thyroid weight. Histopathology: Liver: ↑ hepatocellular vacuolation, mainly diffuse periportal (in 4/10 m versus 2/10 control m). Thyroid: ↑ ultimo-branchial cysts (in 8/10 m versus 4/10 control m). **Females:** No adverse effects. *No LOAEL set for the recovery group*
2 year dietary Rat: Wistar 60/sex/dose Penflufen purity 95.6% Microscopic examination carried out in all organs in all dose groups. OECD 453 GLP: Yes DAR B.6.5.1 Unpublished Study (ref). (2009)	0, 100, 2000, 7000 ppm corresponding to 0, 4.0/5.6, 79/113, 288/399 mg/kg bw/day. **STOT RE guidance value for in rat 2 year study is ≤ 12.5 mg/kg bw/day, calculated from the guidance value for a 90 day study.**	***Non-neoplastic findings (neoplastic findings in the 2 year rat study are reported in section 4.9):*** **100 ppm (4.0/5.6 mg/kg bw/day)** **Males:** Histopathology (non-neoplastic): Liver: - hepatocellular hypertrophy, panlobular to centrilobular (5/60 compared to 0/60 in control m) - eosinophilic foci of cellular alteration (30/60 compared to 23/60 in control m) - interstitial focal mononuclear cell infiltrate (32/60 compared to 28/60 in control m) **Females:** Histopathology (non-neoplastic): Liver: - eosinophilic foci of cellular alteration (38/60 compared to 27/60 in control f). -

		2000 ppm (79/113 mg/kg bw/day) **Males:** Clinical chemistry: ↓ bilirubin 43% (max decrease) Histopathology (non -neoplastic): Liver: - hepatocellular macrovacuolation, diffuse, mainly centrilobular (in 9/60 animals compared to 0/60 in control m) - hepatocellular hypertrophy, panlobular to centrilobular (21/60 compared to 0/60 in control m) - focal brown pigment (9/60 compared to 0 in control m) - eosinophilic foci of cellular alteration (32/60 compared to 23/60 in control m) - interstitial focal mononuclear cell infiltrate (36/60 compared to 28/60 in control m) Thyroid: - diffuse follicular hypertrophy (1/60 compared to 0 in control m) - colloid alteration (30/60 compared to 25/60 in control m) **Females:** ↓ bodyweight gain (11% at week 102), ↓food consumption. Clinical chemistry: ↓ bilirubin 44% (max decrease), ↑ cholesterol 16%. Histopathology (non -neoplastic): Liver: - hepatocellular macrovacuolation, diffuse, mainly centrilobular (in 18/60 versus 0/60 in control f) - hepatocellular hypertrophy, panlobular to centrilobular (22/60 versus 0/60 in control f) - focal brown pigment (18/60 versus 0 in control f) - eosinophilic foci of cellular alteration (46/60 versus 27/60 in control f) - interstitial focal mononuclear cell infiltrate (40/60 versus 30/60 in control f). Thyroid: - colloid alteration (17/60 compared to 2/60 in control f). **7000 ppm (288/399 mg/kg bw/day)** **Males:** ↓ bodyweight gain (5% at week 102). Organ weights: ↑ absolute/relative liver weight (14/17%) Haematology: ↓ reticulocytes 57% (max decrease), ↓ % reticulocytes 69% (max decrease). Clinical chemistry: ↓ bilirubin 53% (max decrease). Histopathology (non- neoplastic): Liver: - hepatocellular macrovacuolation ,diffuse, mainly centrilobular (in 23/60 animals versus 0/60 in control m) - hepatocellular hypertrophy, panlobular to centrilobular (50/60 versus 0/60 in control m) - focal brown pigment (23/60 versus 0 in control m) - eosinophilic foci of cellular alteration (30/60 versus 23/60 in control m) - interstitial focal mononuclear cell infiltrate (36/60 versus 28/60 in control m) Thyroid: - diffuse follicular hypertrophy (3/60 versus 0 in control m) - colloid alteration (48/60 versus 25/60 in control m). **Females:** ↓ bodyweight gain (18% at week 102), ↓food consumption. Organ weights: ↑ relative liver weight (13%)

		Haematology: ↓ reticulocytes 19% (max decrease), ↓ % reticulocytes 21% (max decrease). Clinical chemistry: ↓ bilirubin 64% (max decrease), ↑ cholesterol 26% (max increase). Histopathology (non -neoplastic): Liver: - hepatocellular macrovacuolation, diffuse, mainly centrilobular (in 30/60 animals versus 0/60 in control f) - hepatocellular hypertrophy, panlobular to centrilobular (47/60 versus 0/60 control f) - focal brown pigment (30/60 versus 0 in control f) - eosinophilic foci of cellular alteration (39/60 versus 27/60 in control f) - interstitial focal mononuclear cell infiltrate (40/60 versus 30/60 in control f) Thyroid: - diffuse follicular hypertrophy (3/60 versus 0 in control f) - colloid alteration (29/60 versus 2/60 in control f). Ovary: - tubulostromal hyperplasia (7/60 f versus 3/60 in control f) *LOAEL Non-neoplastic 100 ppm (4.0/5.6 mg/kg bw/day)*
28 day oral dietary Mouse: C57BL/6J 5/sex/dose Penflufen purity 98.6% Similar to OECD 407 GLP: No DAR 6.3.2 Unpublished Study (ref). (2005)	0, 150, 3500, 7000 ppm corresponding to 0, 26/31, 632/741, 1274/1585 mg/kg bw/day in m/f **STOT RE guidance value is ≤ 300 mg/kg bw/day based on values for the rat 28 day study.**	There were no deaths or clinical signs of toxicity at any dose. **150 ppm (26/31 mg/kg bw/day)** No adverse effects. **3500 ppm (632/741 mg/kg bw/day)** **Males:** Organ weights: ↑ relative liver weight (14%) Clinical chemistry: 52% ↓ cholesterol **Females:** Organ weights: ↑ absolute and relative liver weight (24/32%) Clinical chemistry: 51% ↓ cholesterol. **7000 ppm (1274/1585 mg/kg bw/day)** **Males:** Clinical chemistry: ↓ cholesterol (58%) Organ weights: ↑ absolute and relative liver weight (20/24%) Histopathology: diffuse hepatocellular hypertrophy (in 1/5 m versus 0 in control m). **Females:** Organ weights: ↑ absolute and relative liver weight (28/28%), diffuse hepatocellular hypertrophy (in 3/5 f versus 0 in control f). Clinical chemistry: ↓ cholesterol (44%), ↑ alkaline phosphatase (32%). *LOAEL 3500 ppm (632/741 mg/kg bw/day)*

90 day oral dietary Mouse: C57BL/6J 10/sex/dose Penflufen purity 98.8% OECD 408 GLP: Yes DAR 6.3.2 Unpublished Study (ref). (2006c)	0, 150, 3500, 7000 ppm corresponding to 0, 26.9/31.5, 638/757, 1238/1600 mg/kg bw/day in m/f **STOT RE guidance value is ≤ 100 mg/kg bw/day, based on the guidance value for a 90 day study in the rat.**	There were no deaths or clinical signs of toxicity at any dose. **150 ppm (26.9/31.5 mg/kg bw/day)** No adverse effects. **3500 ppm (638/757 mg/kg bw/day)** **Males:** Organ weights: ↑ relative liver weight (16%) Clinical chemistry: 35% ↓ cholesterol Histopathology: centrilobular hepatocellular hypertrophy (in 4/10 animals compared with 1/10 in control m) **Females:** Organ weights: absolute and relative liver weight (13/16%) Clinical chemistry: 57% ↓ cholesterol Histopathology: centrilobular hepatocellular hypertrophy (in 4/10 f versus 0 in control f) **7000 ppm (1238/1600 mg/kg bw/day)** **Males:** Organ weight: ↑ absolute and relative liver weight (20/23%) Clinical chemistry: 45% ↓ cholesterol Histopathology: diffuse hepatocellular hypertrophy (in 9/10 m versus 1/10 in control m). **Females:** Organ weights: ↑ absolute and relative liver weight (33/32%) Clinical chemistry: 60% ↓ cholesterol Histopathology: diffuse hepatocellular hypertrophy (in 7/10 f versus 0 in control f). *LOAEL 3500 ppm (638/757 mg/kg bw/day)*
1 year dietary Mouse: C57BL/6J 10/sex/dose Penflufen purity 95.6% This study was conducted as part of the carcinogenicity study in mice. No clinical chemistry and no histopathology were performed. OECD 451 GLP: Yes DAR B.6.5.2 Unpublished Study (ref). (2009a)	0, 100, 2000, 6000 ppm corresponding to 0, 14.5/18.8, 148/187, 891/1137 mg/kg bw/day. **STOT RE guidance value is ≤ 25 mg/kg bw/day, calculated from the guidance value for a 90 day study in the rat.**	There were no treatment-related effects on survival at any dose. **100 ppm (14.5/18.8 mg/kg bw/day)** No adverse effects **2000 ppm (148/187 mg/kg bw/day)** No adverse effects **6000 ppm (891/1137 mg/kg bw/day)** **Males:** Organ weights: ↑ absolute/relative liver weight (11%/9%) **Females:** Organ weights: ↑ relative liver weight (21%) *No LOAEL set for chronic phase of this study*

18 month dietary Mouse: C57BL/6J 50/sex/dose Penflufen purity 95.6% OECD 451 GLP: Yes DAR B.6.5.2 Unpublished Study (ref). (2009a)	0, 100, 1000, 6000 ppm corresponding to 0, 14.3/18.4, 146/182, 880/1101 mg/kg bw/day. **STOT RE guidance value is c.a., ≤ 16.7 mg/kg bw/day, calculated from the guidance value for a 90 day study in the rat.**	***Non-neoplastic findings (neoplastic findings in the 78 week mouse study are reported in section 4.9):*** **100 ppm (14.3/18.4 mg/kg bw/day)** **Males:** Histopathology: Liver: centrilobular hepatocellular hypertrophy (13/49 m versus 0 in control m) **2000 ppm (146/182 mg/kg bw/day)** **Males:** Histopathology: Liver: centrilobular hepatocellular hypertrophy (29/49 m versus 0 in control m) **Females:** Histopathology: Liver: centrilobular hepatocellular hypertrophy (5/50 f versus 0 in control f) **6000 ppm (880/1101 mg/kg bw/day)** **Males:** Organ weights: ↑ absolute/relative liver weight (19%/20%) Histopathology: Liver: - centrilobular hepatocellular hypertrophy (46/48 m versus 0 in control m) - diffuse hepatocellular vacuolation (19/48 m versus 10/48 in control m) **Females:** Organ weights: ↑ absolute/relative liver weight (23%/24%) Histopathology: Liver: - centrilobular hepatocellular hypertrophy (31/50 f versus 0 in control f) - periportal diffuse hepatocellular vacuolation (41/50 f versus 14/50 in control f) Thyroid: focal/multifocal follicular cell hyperplasia (38/50 f versus 23/50 in control f) *LOAEL non neoplastic 1000 ppm (14.3/18.4 mg/kg bw/day)*

28 day oral dietary Beagle dogs 2/sex/dose Penflufen purity 98.8% Non-guideline GLP: No DAR 6.3.3 Unpublished Study (ref). (2005)	0, 1300, 6500, 26,000 ppm corresponding to 0, 49/52, 244/246, 759/895 mg/kg bw/day in males/females **STOT RE guidance value of ≤ 300 mg/kg bw/day is considered relevant, calculated from the guidance value for a 90 day study in the rat.**	There were no deaths or clinical signs of toxicity at any dose. **1300 ppm (49/52 mg/kg bw/day)** No adverse effects. **6500 ppm (244/246 mg/kg bw/day)** **Males:** ↓ bodyweight gain & food consumption Organ weights: ↑ absolute and relative liver weight. Clinical chemistry: ↑ alkaline phosphatase Histopathology: Liver: centrilobular hepatocellular hypertrophy, Thyroid: follicular cell hypertrophy & decreased follicular diameter (versus 0 histopathological findings in control m) **Females:** ↓ bodyweight gain & food consumption Organ weights: ↑ absolute and relative liver weight. Clinical chemistry: ↑ alkaline phosphatase Histopathology: Liver: centrilobular hepatocellular hypertrophy (versus 0 in control f) Thyroid: follicular cell hypertrophy & decreased follicular diameter (versus 0 in control f) **26,000 ppm (759/895 mg/kg bw/day)** **Males:** ↓ bodyweight gain & food consumption Organ weights: ↑ absolute and relative liver weight Clinical chemistry: ↑ alkaline phosphatase, ↓ cholesterol. Histopathology: Liver: centrilobular hepatocellular hypertrophy (versus 0 in control m) Thyroid: follicular cell hypertrophy & decreased follicular diameter (versus 0 in control m) **Females:** ↓ bodyweight gain & food consumption Organ weights: ↑ absolute and relative liver weight Clinical chemistry: ↑ alkaline phosphatase, ↓ cholesterol Histopathology: Liver: centrilobular hepatocellular hypertrophy (versus 0 in control f) Thyroid: follicular cell hypertrophy & decreased follicular diameter (versus 0 in control f) *LOAEL 6500 ppm (244/246 mg/kg bw/day)*

90 day oral dietary Beagle dogs 4/sex/dose Penflufen purity 95.6% OECD 409 GLP: Yes DAR 6.3.3 Unpublished Study (ref). (2008)	0, 180, 1800, 18,000 ppm corresponding to 0, 5.6/6.1, 55.7/63.1, 532/568 mg/kg bw/day in m/f **STOT RE guidance value of ≤ 100 mg/kg bw/day is considered relevant, based on the guidance value for a 90 day study in the rat.**	There were no deaths or clinical signs of toxicity at any dose. **180 ppm (5.6/6.1 mg/kg bw/day)** No adverse effects. **1800 ppm (55.7/63.1 mg/kg bw/day)** **Males:** Liver: Diffuse panlobular hepatocellular hypertrophy 1/4 m compared with 0 in control m). **Females:** Liver: Diffuse panlobular hepatocellular hypertrophy (in 3/4 f compared with 0 in control f). **18,000 ppm (532/568 mg/kg bw/day)** **Males:** Organ weights: ↑ absolute and relative liver weight (36/37%), ↑ absolute adrenal weight (50%) Clinical chemistry: ↑ alkaline phosphatase (4 times higher than controls), 67% ↑ cholesterol. Histopathology: Liver: diffuse panlobular hepatocellular hypertrophy (in 4/4 m versus 0 in control m), multifocal intrahepatocellular eosinophilic material (in 2/4 m versus 0 in control m) and hepatic perilobular multifocal single cell death (in 2/4 m versus 0 in control m) Adrenals: diffuse cortical hypertrophy/hyperplasia (in 2/4 m versus 0 in control m). **Females:** ↓ bodyweight gain (82%) & food consumption Organ weights: ↑ relative liver weight (50%) Haematology: 46% ↑ platelet count Clinical chemistry: ↑ alkaline phosphatase (4 times higher than controls). Histopathology: Liver: diffuse panlobular hepatocellular hypertrophy (in 4/4 f versus 0 in control f), multifocal intrahepatocellular eosinophilic material (in 1/4 f versus 0 in control f) and hepatic perilobular multifocal single cell death (in 1 f versus 0 in control f). *LOAEL 1800 ppm (55.7/63.1 mg/kg bw/day)*

1 year oral dietary Beagle dogs 4/sex/dose Penflufen purity 95.6% OECD 452 GLP: Yes DAR 6.3.3 Unpublished Study (ref). (2009)	0, 200, 1000, 10,000 ppm corresponding to 6.8/7.7, 32/38, 357/425 mg/kg bw/day in males/females. **STOT RE guidance value of ≤25 mg/kg bw/day is considered relevant, calculated from the guidance value for the 90 day study in the rat.**	There were no deaths or clinical signs of toxicity at any dose. **200 ppm (6.8/7.7 mg/kg bw/day)** No adverse effects. **1000 ppm (32/38 mg/kg bw/day)** **Males:** Histopathology: intrahepatocellular brown pigment (in 1 m versus 0 in control m) **Females:** Organ weights: ↑ absolute and relative liver weight (17/28%) Histopathology: panlobular hepatocellular hypertrophy (in 1 f versus 0 in control f), ↓ hepatocellular glycogen accumulation (in 1 f) **10,000 ppm (357/425 mg/kg bw/day)** **Males:** ↓ food consumption Organ weights: ↑ absolute and relative liver weight (28/32%) Clinical chemistry: ↑ alkaline phosphatase (2.5 times higher than control m). Histopathology: Liver: panlobular hepatocellular hypertrophy (in 3 males compared to none in control m), intrahepatocellular brown pigment (in 2/4 males compared to none in control m), ↓ hepatocellular glycogen accumulation (in 3 m) Thyroid: follicular cell hypertrophy (in 1 male compared with none in control m) **Females:** ↓ bodyweight gain (54%) Organ weights: ↑ absolute and relative liver weight (25/51%) Clinical chemistry: ↑ alkaline phosphatase (up to 7 times higher than controls). Histopathology: Liver: panlobular hepatocellular hypertrophy (in 4/4 females compared with none in control f), intrahepatocellular brown pigment (in 3 females compared with none in control f), ↓ hepatocellular glycogen accumulation (in 3 f) Thyroid: follicular cell hypertrophy (in 3/4 f versus 1/4 in control f) *LOAEL* *Males 10,000 ppm (357 mg/kg bw/day)* *Females 1000 ppm (38 mg/kg bw/day)*

4.6.1 Non-human information

4.6.1.1 Repeated dose toxicity: oral

Rats

In a 28 day study in the rat the target organ was the liver. The only effects below the guidance value for classification (i.e., at 154 mg/kg bw/day) included an increase in relative liver weight in males (11%) which was accompanied by an increase in enzyme activity (cytochrome P450, BROD and PROD). Increased enzyme activity was also noted in females at doses below the guidance value for classification (169 mg/kg bw/day), but an increase in relative liver weight was not observed until the higher dose level of 648 mg/kg bw/day (19% increase in relative liver weight in females and rising to 26% in males). In both males and females an increased incidence of centrilobular hepatocyte hypertrophy was noted at 560/648 mg/kg bw/day respectively.

In a 29/30 day immunotoxicity study no effects were observed at doses relevant for classification. At higher doses, only decreased body weight gain and increased food consumption were observed.

In the first 90-day study, the only effects observed at doses below the guidance value for classification (i.e., at 9.5 mg/kg bw/day) included an increase in relative liver weight (11%) in males and exocrine single cell necrosis in the pancreas in 5/10 males compared to 0/10 in controls. However, this latter effect was only observed in 4/10 males in both the two higher dose groups (457 and 949 mg/kg bw /day) and in 4/10 females at the highest dose of 1009 mg/kg bw/day only. The grading of the lesion in the histopathology report was 'minimal' or 'slight' and the incidence was within the laboratory historical controls. Increased relative liver weights were only observed in females (26%) from 492 mg/kg bw/day. Centrilobular hepatocyte hypertrophy was observed in males and females from doses of 457/494 mg/kg bw/day respectively. In addition an increased incidence of thyroid follicular cell hypertrophy was observed in males and females, but only from doses of 457 and 1009 mg/kg bw/day respectively.

In a second 90-day study, no adverse effects were noted at doses relevant for classification. At higher doses, increases in relative liver weight were observed (13% and 23% in males at 126 and 576 mg/kg bw/day and 12% and 28% in females at 156 and 609 mg/kg bw/day). Exocrine single cell necrosis in the pancreas was noted in males only, but was only marginally higher than in the control group (incidence was 2, 3, 3, 4 all out of 10 in the 0, 3.2, 9.3, 228 mg/kg bw/day dose groups respectively). The grading of the lesion ranged from 'minimal' to 'slight' and was within the laboratory historical controls.

In the longer term studies, the only effects seen at doses relevant for classification were increased hepatocellular hypertrophy and eosinophlic foci of cellular alteration in males at 4 mg/kg bw/day and eosinophilic foci of cellular alterations in females from 5.6 mg/kg bw/day in the 2-year study. At higher doses i.e., 79/113 mg/kg bw/day in m/f respectively in the 2-year study, increased hepatocellular macrovacuolation and thyroid follicular cell hypertrophy were noted. Increased relative liver weights were only noted in males and females at doses of 288 and 399 mg/kg bw/day respectively. Exocrine single cell necrosis was observed in males in the one year rat study in the top dose group (327 mg/kg bw/day), but the pancreas was not examined in the low and mid dose groups. It is noted however, that this finding was also seen in the control group (4/10 males). This finding was not observed in the 2-year study with a top dose of 288 and 399 mg/kg bw/day in males and females respectively.

Mice

In the 28-day study no adverse effects were noted at doses relevant for classification. Increased relative liver weight was noted in males and females from doses of 632 and 741 mg/kg bw/day

respectively. A small increase in the incidence of hepatocellular hypertrophy was noted from 1274 and 1585 mg/kg bw/day in males and females respectively.

In the 90-day study no adverse effects were noted at doses relevant for classification. Increase liver weights in males (16%) and in females (16%) were observed from doses of 638 and 757 mg/kg/day respectively, along with an increased incidence of hepatocellular hypertrophy.

In the longer term studies, hepatocellular hypertrophy was observed from doses of 146/182 in m/f respectively.

Dogs

In the 28-day study, an increased incidence of hepatocellular hypertrophy and increased relative liver weight were observed from doses of 244 and 246 mg/kg bw/day in males and females respectively. Thyroid, follicular cell hypertrophy was also observed in males and females at this dose level.

In the 90-day study, an increased incidence of hepatocellular hypertrophy was observed from a dose level of 55.7/63.1 mg/kg bw/day m/f. At the higher dose level (532/568 mg/kg bw/day) increased relative liver weight was observed in males (37%) and females (50%). In addition, increased intrahepatocellular eosinophilic material and hepatic perilobular single cell death were also noted at this dose level in males and females.

In the 1-year study an increased incidence of hepatocellular hypertrophy was noted in females from a dose of 38 mg/kg bw/day along with an increased relative liver weight of 28%. This was only observed in males from the higher dose level of 357 mg/kg bw/day. In both males and females an increased incidence of thyroid follicular cell hypertrophy was also noted in the high dose group.

4.6.1.2 Repeated dose toxicity: inhalation

No data available.

4.6.1.3 Repeated dose toxicity: dermal

Table 15: Summary table of relevant repeated dose dermal toxicity studies

Method	Dose Levels	Observations and Remarks (main toxicological effects)
28 day dermal Rat: Wistar Hanover 10/sex/dose Penflufen purity 95.6% Topical application onto a gauze pad moistened with water. OECD 410 GLP: Yes DAR 6.3.4 Unpublished Study (ref). (2009)	0, 100, 300, 1000 mg/kg bw/day 6 hour daily exposure, 5 days a week for 4 weeks equating to a total of 21 doses. **STOT RE guidance value in rat 28 day dermal study is ≤ 600 mg/kg bw/day**	**100 mg/kg bw/day** No adverse effects. **300 mg/kg bw/day** No adverse effects. **1000 mg/kg bw/day** Thymus: Increased lymphocyte debris within the thymic cortices in 7/10 m and 7/10 f versus 0 in controls. *LOAEL* *1000 mg/kg bw/day in males and females*

(The LOAEL is provided for information only; it was taken from the EFSA peer review).

In a standard GLP and guideline compliant 28 day study 10 rats/sex/dose were administered penflufen by dermal administration at a dose of 0, 100, 300 or 1000 mg/kg bw/day for 6 hours, 5 days a week. Penflufen was applied to a gauze pad moistened with water and secured to a shaved area of the trunk. Blood samples were taken at the end of the study and a range of parameters measured. At necropsy the weights of major organs were recorded. All organs from the control and high dose animals, together with the thymus and cervical lymph nodes from the 300 mg/kg/day group, were subjected to microscopic examination.

There were no treatment-related deaths. No signs of local effects or clinical signs indicative of systemic toxicity were observed. There were no adverse findings in the low and mid dose group. In the top dose group (1000 mg/kg/d) histopathological changes were seen in the thymus of 7 males and 7 females as evidenced by increased thymic debris within the thymic cortices.

4.6.1.4 Repeated dose toxicity: other routes

No data available.

4.6.1.5 Human information

No data available.

4.6.2 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

The repeat dose toxicity of penflufen via the oral route was investigated in rats, mice and dogs. In addition, toxicity via the dermal route was investigated in rats. No repeated dose inhalation studies on penflufen are available.

The liver effects comprised increased relative and absolute weights, centrilobular hepatocellular hypertrophy and increased liver-enzyme activity. Liver effects observed at doses below the guidance cut-off values for classification as STOT-RE, were minimal and thus are not relevant for classification. In the two-year rat study increased incidence and severity of eosinophilic foci of hepatocellular alteration were seen in males and females at doses relevant to classification. Although in females these findings slightly exceeded the historical control incidence they were a very common lesion also in the controls and were not accompanied by any significant increase in liver weight so are not considered to be evidence of a severe or significant adverse effect on the liver.

Another target organ was the thyroid. In the 28-day dog study, thyroid follicular cell hypertrophy and decreased follicular diameter were observed from 244 mg/kg/d (only two animals/sex/dose); such effects were not reported at doses relevant for classification in the 90-day nor one-year dog studies. Thyroid effects in rats (follicular cell hypertrophy, focal/multifocal colloid alteration, an increase in ultimo-branchial cysts) were only reported at doses above the guidance values for classification. There were no effects on the thyroid in the mouse studies. Since findings in the thyroid at doses relevant to classification are only seen in the 28-day dog study and based on only two animals per sex per dose group this is considered insufficient evidence of a severe or significant adverse effect on the thyroid and are not considered further.

Exocrine single cell necrosis was reported in the pancreas of male rats at all doses and in females of the high-dose group in a 90-day rat study (Unpublished Study (ref). 2006a). However, a steep dose-response relationship was not evident and the grading of the lesion in the histopathology report was 'minimal' or 'slight'. A second 90 day rat study (Unpublished Study (ref). 2006b) used lower doses to further investigate the findings in the pancreas and found that incidence of exocrine single cell necrosis in males was only marginally higher than in the control group (incidence was 2, 3, 3, 4 in the 0, 3.2, 9.3, 228 mg/kg bw/day dose groups respectively). The grading of the lesion ranged from 'minimal' to 'slight', and there was no increase in this lesion in any treated females. The findings in both of these 90 day studies were within the historical control range for the same laboratory and strain. Exocrine single cell necrosis also occurred in males in the one year rat study (Unpublished Study (ref). 2009) in the top dose group (327 mg/kg bw/day). This finding is above the guidance value for classification, but the pancreas was not examined in the low and mid dose groups so it is not possible to conclude whether any findings occurred at lower doses in the one year study. It is noted however, that this finding was also seen in the control group (4/10 males). In the two year rat study (Unpublished Study (ref). 2009) using 60 animals per sex/dose group, with a top dose of 288 and 399 mg/kg bw/day in males and females respectively, all animals were subject to a full histopathological examination and no treatment-related findings were detected in the pancreas in any of the treated groups. Further, no findings in the pancreas were reported in either the mouse or dog. Taking a weight of evidence approach it is concluded that the pancreas findings in the 90 day and 1 year rat studies are isolated findings, and do not support classification for repeated-dose toxicity.

4.6.3 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Substances are classified for repeated-dose toxicity when they cause significant or severe health effects that impair function of an identified target organ, or if they cause generalised changes of a less severe nature involving several organs. These effects should generally occur below the oral guidance value of 100 mg/kg bw/day (for a classification in category 2) obtained in a 90-day rat study. The oral guidance value for a classification in category 1 is ≤ 10 mg/kg bw/day. The equivalent guidance values for a 28-day study are ≤ 300 mg/kg bw/day and ≤ 30 mg/kg bw/day, respectively.

In the oral studies, the most sensitive target organ was the liver. Changes in the liver were seen in all species but in most cases these findings occurred at doses that were higher than the relevant guidance value for classification for STOT RE; the exception being the 28-day rat and 28-day dog studies. In the 28-day rat study, liver effects at 154/169 mg/kg bw/day (in males/females) included an increase in relative liver weight (11%) in males only and an increase in enzyme activity (cytochrome P450, BROD and PROD) in both males and females. In the 28-day dog study, liver effects at 244/246 mg/kg bw/day (in males/females) included increased absolute and relative liver weight accompanied by centrilobular hepatocellular hypertrophy in both sexes as well as increased alkaline phosphatase which is often an indicator of liver damage; however, the very small group sizes in this study and lack of reproducibility of this finding is not robust evidence of an adverse effect on the liver. Overall, it is concluded that the liver effects at doses below the guidance values were minimal and there was no consistent or conclusive evidence of hepatotoxicity. Therefore, they are not considered to support classification for STOT-RE.

Exocrine single cell necrosis was reported in the pancreas and in one 90 day rat study was observed in males only at a dose level relevant for classification as STOT RE 1. These findings were more were not dose related, were within the range of the laboratory historical controls and, moreover were not reproducible in a second 90-day study. The same finding was noted in male rats in the 1 year study, but there was no evidence of damage to the pancreas in the 2 year rat study at doses up to 288/399 mg/kg bw/day in m/f respectively. Further, no findings in the pancreas were reported in the mouse or dog studies. Overall, it is concluded that the effects seen in the rat studies were likely to be incidental and do not indicate a severe or significant toxic effect in the pancreas. Therefore, they are not considered to support classification for STOT-RE.

In the 28 day repeat dose dermal study in the rat, the only adverse effects related to mild histopathological changes in the thymus at the top dose of 1000 mg/kg bw/day which is above the guidance value for classification (i.e., 600 mg/kg bw/day) for STOT-RE.

Overall, it is concluded that penflufen does not meet the criteria for classification for repeated-dose toxicity (STOT-RE).

4.6.4 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE.

Not classified - conclusive but not sufficient for classification

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 Year: 2004	Non-guideline Non-GLP Doses: 0, 150, 2000, 7000 ppm; 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*-dependentylation; BROD = benzyloxyresorufin *O*-debenzylation)	560/648 mg/kg bw/d (above GV): • ↓ bw gain (f) • ↑ liver wt (relative, by 26%/19% m/f) • 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	300 mg/kg bw/d

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) • ↑ 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)	100 mg/kg bw/d

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) 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	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) • 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:	25 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 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)	12.5 mg/kg bw/d

		• 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 sacrifice after 12 months omitted here because of the lack of	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) 50/sex/dose	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) • 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	16.7 mg/kg bw/d

histophath. investigations)		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 Year: 2009	OECD TG 452 GLP Doses: 0, 200, 1000, 10000 ppm; corresponding to 0, 6.8/7.7, 32/38,	357/425 mg/kg bw/d (above GV): • ↓ bw gain (f) • ↑ liver wt (relative by 32%/51% m/f) • ↑ ALP (up to 4/7-fold m/f) • Panlobular hepatocellular hypertrophy	25 mg/kg bw/d

	357/425 mg/kg bw/d (m/f) 4/sex/dose	• 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.

4.7 Germ cell mutagenicity (Mutagenicity)

Six standard *in vitro* tests and one standard *in vivo* test are available and summarised in Table 16 below.

Table 16: Summary table of relevant in vitro and in vivo mutagenicity studies

In Vitro Data				
Method	**Organism/strain**	**Concentrations tested**	**Result**	**Reference**
Bacterial reverse mutation assay (Ames) Plate incorporation and pre-incubation methods Penflufen purity 95.6% OECD 471 (1997) GLP Yes	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	From 16 µg/plate up to the test limit concentration of 5000 µg/plate	Negative ±S9. Toxicity to the bacteria and/or precipitation occurred at concentrations of 500 µg/plate	DAR 6.4.1 Unpublished Study (ref). (2007a)
mutation assay (Ames) Plate incorporation and pre-incubation methods Penflufen purity 94.4% OECD 471 (1997) GLP Yes	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	From 3 µg/plate up to the test limit concentration of 5000 µg/plate	Negative ±S9. Toxicity to the bacteria and/or precipitation occurred at concentrations of 500 µg/plate.	DAR 6.4.1 Unpublished Study (ref). (2009)
Mammalina cell chromosome aberration test Penflufen purity 95.6% OECD 473 GLP YES	Chinese hamster V79 cells	Experiment 1: cultures were exposed to penflufen for 4 hours at 20, 40, 70 µg/mL without S9 mix and 30, 60, 90 µg/ml with S9 mix. Experiment 2: cultures were exposed to penflufen for 18 hours at 3, 6 and 12 µg/ml without S9 mix.	Negative ±S9. Doses chosen were based on a reduction in mitotic index in a preliminary test.	DAR 6.4.1 Unpublished Study (ref). (2007)
Mammalian cell chromosome aberration test Penflufen purity 94.4% OECD 473 GLP Yes	Chinese hamster V79 cells	Experiment 1: cultures were exposed to penflufen for 4 hours at 9.4, 18.8, 37.5 µg/mL without S9 mix and 18.8, 37.5, 75.0 µg/ml with S9 mix. Experiment 2: cultures were exposed to penflufen for 18 hours at 4.7, 9.4, 18.8 µg/ml without S9 mix.	Negative ±S9. Doses chosen were based on a reduction in mitotic index or precipitation.	DAR 6.4.1 Unpublished Study (ref). (2009)

Mammalian cell gene mutation test (HPRT locus) Penflufen purity 95.6% OECD 476 GLP Yes	Chinese hamster V79 cells	Cultures were exposed for 5 hours to 12.5 to 150 µg/mL penflufen.	Negative ±S9. Doses were chosen based on a preliminary cytotoxicity assay.	DAR 6.4.1 Unpublished Study (ref). (2007)
Mammalian cell gene mutation test (HPRT locus) Penflufen purity 94.4% OECD 476 GLP Yes	Chinese hamster V79 cells	Cultures were exposed for 4 hours to 7.5 to 125 µg/mL penflufen.	Negative ±S9. Doses were chosen based on a preliminary cytotoxicity assay.	DAR 6.4.1 Unpublished Study (ref). (2009)
In vivo Data				
Method	**Organism/strain**	**Concentrations tested**	**Result**	
Bone marrow micronucleus test Penflufen purity 95.6% Animals were killed 24 hours after the second dose of penflufen for bone marrow sampling OECD 474 GLP Yes	Mouse, NMRI, male, 5 per dose group	Two intraperitoneal doses of penflufen administered on consecutive days at 250, 500, 1000 mg/kg bw/day.	Negative. Doses were chosen on the basis of a preliminary study in which mortalities were observed at 2000 mg/kg bw/day. Clinical signs of toxicity were observed at all dose levels and included apathy, roughened fur, weight loss, sternal recumbency, spasm, difficulty in breathing and slitted eyes. There was an increase in NCEs to PCEs in treated groups suggesting the test substance reached the bone marrow.	DAR 6.4.2 Unpublished Study (ref). (2007b)

4.7.1 Non-human information

4.7.1.1 In vitro data

All tests were negative.

4.7.1.2 In vivo data

Bone marrow micronucleus test

A GLP and guideline-compliant reliable study conducted in mice was negative for increases in micronucleated immature erythrocytes.

4.7.2 Human information

No information available.

4.7.3 Other relevant information

No information available.

4.7.4 Summary and discussion of mutagenicity

Data indicate that penflufen is not mutagenic *in vitro* and *in vivo.*

4.7.5 Comparison with criteria

No classification for mutagenicity is required.

4.7.6 Conclusions on classification and labelling

Not classified - conclusive but not sufficient for classification

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 required.

4.8 Carcinogenicity

The chronic toxicity and carcinogenic potential of penflufen has been investigated in rats and mice. Several studies have also been conducted to investigate the mode of action and relevance to humans.

Table 17: Summary table of relevant carcinogenicity studies

(The values for LOAEL are provided for information only. They have been agreed at the EFSA Pesticide Peer Review Meeting.)
All historical control data are from the same lab and strain of animal and dated within 5 years of the current studies.

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td>Rat (Wistar)
60/sex/dose
(80 up to week 52)

2 year dietary

Date performed Jan 2007 – Feb 2009

Penflufen purity 95.6%

Microscopic examination carried out in all organs in all dose groups in the main study.

OECD 453
GLP: Yes

DAR 6.5.1 Unpublished Study (ref). (2009)</td><td>0, 100, 2000, 7000 ppm corresponding to 0, 4.0/5.6, 79/113, 288/399 mg/kg bw/day.</td><td><u>Non-neoplastic findings:</u>
See Table 14 for full details of non-neoplastic findings.

<u>Survival:</u>
Treatment had no adverse effect on survival up to highest dose tested.
Survival was lower in males than females but is considered adequate to assess the carcinogenicity response.
<table>
<tr><th></th><th colspan="4">Males</th><th colspan="4">Females</th></tr>
<tr><td>Dose (ppm)</td><td>0</td><td>100</td><td>2000</td><td>7000</td><td>0</td><td>100</td><td>2000</td><td>7000</td></tr>
<tr><td>Start of week 52</td><td>80/80</td><td>79/80</td><td>78/80</td><td>78/80</td><td>76/80</td><td>80/80</td><td>78/80</td><td>77/80</td></tr>
<tr><td>Death due to accident or anaesthesia</td><td>0</td><td>0</td><td>4</td><td>2</td><td>2</td><td>1</td><td>1</td><td>0</td></tr>
<tr><td>Start of week 97</td><td>31/60</td><td>32/60</td><td>30/60</td><td>35/60</td><td>40/60</td><td>45/60</td><td>50/60</td><td>43/60</td></tr>
<tr><td>Start of week 104</td><td>25/60</td><td>25/60</td><td>23/60</td><td>25/60</td><td>31/60</td><td>37/60</td><td>44/60</td><td>43/60</td></tr>
<tr><td>At scheduled kill</td><td>19/60</td><td>24/60</td><td>21/60</td><td>24/60</td><td>29/60</td><td>37/60</td><td>43/60</td><td>43/60</td></tr>
</table>
<u>Bodyweight and food consumption:</u>
100 ppm (4.0/5.6 mg/kg bw/day): No effect in either sex
2000 ppm (79/113 mg/kg bw/day): No effect in males. In females ↓ bodyweight gain (11% at week 102), ↓food consumption
7000 ppm (288/399 mg/kg bw/day): In males ↓ bodyweight gain (5% at week 102). In females ↓ bodyweight gain (18% at week 102), ↓food consumption.

<u>Tissue specific findings:</u>
Liver was most sensitive target organ: increased liver weight, histopathological findings and clinical chemistry changes.
Histopathological findings also seen in the ovary and thyroid, and there were some minimal haematological changes.</td></tr>
</table>

Neoplastic findings (includes all animals from terminal kill and those that died during the course of the study):

Liver:

Dose (ppm)	0	100	2000	7000	Historical control incidence (50 – 60 animals per control group)
Number of animals	60	60	60	60	
Males: Hepatocellular adenoma	1	1	0	2	10 studies Range: 0 – 3 (0 – 5%) Overall incidence: 14/585 (2.4%)
Males: Hepatocellular carcinoma	1	1	0	0	
Females: Hepatocellular adenoma	0	2	5*	4	10 studies Range: 0 – 3 (0 – 5%) Overall incidence: 11/585 (1.9%)
Females: Hepatocellular carcinoma	0	0	1	0	

* significantly different from control, p≤0.05

Ovary:

Dose (ppm)	0	100	2000	7000	Historical control incidence (50 – 60 animals per control group)
Number of animals	60	60	60	60	
Ovary: tubulostromal adenocarcinoma	0	1	1	0	
Ovary: tubulostromal adenoma	2	1	1	7	10 studies Range: 0 – 4 (0 – 6.7%) Overall incidence: 15/580 (2.6%)

Brain:

Dose (ppm)	0	100	2000	7000	Historical control incidence (50 – 60 animals per control group)
Number of animals	60	60	60	60	
Males: Astrocytoma	1	0	0	3	10 studies Range: 0 – 2 (0 – 3.7%) Overall incidence: 9/584 (1.54%)
Females: Astrocytoma	0	0	0	0	10 studies Range: 0 (0%) Overall incidence 0/585 (0%)

Haematopoietic system:

Dose (ppm)	0	100	2000	7000	Historical control incidence (50 – 60 animals per control group)
Number of animals	60	60	60	60	
Males: Histiocytic sarcoma	0	3	3	5*	9 studies Range: 0 – 2 (0 –3.3%) Overall incidence: 4/525 (1.54%)
Females: Histiocytic sarcoma	3	0	0	0	9 studies Range: 0 – 4 (0 –6.7%) Overall incidence: 6/525 (1.1%)

* significantly different from control, p≤0.05

<table>
<tr>
<td>Mouse C57BL/6J strain
Dietary
50/sex/dose (plus 10 in chronic satellite group killed at 54 weeks)

78 week

Date performed March 2007 – Oct 2009

Penflufen purity 95.6%

OECD 451
GLP: Yes

DAR 6.5.2 Unpublished Study (ref). (2009a)

Microscopic examination carried out in all organs in all dose groups.</td>
<td>0, 100, 1000, 6000 ppm corresponding to 0, 14.3/18.4, 146/182, 880/1101 mg/kg bw/day.</td>
<td>
<u>Non-neoplastic findings:</u>
See Table 14 for full details of non-neoplastic findings.

<u>Survival:</u>
<table>
<tr><th></th><th colspan="4">Males</th><th colspan="4">Females</th></tr>
<tr><td>Dose (ppm)</td><th>0</th><th>100</th><th>2000</th><th>6000</th><th>0</th><th>100</th><th>2000</th><th>6000</th></tr>
<tr><td>Start of week 52</td><td>54/60</td><td>55/60</td><td>58/60</td><td>59/60</td><td>57/60</td><td>57/60</td><td>57/60</td><td>57/60</td></tr>
<tr><td>Start of week 80</td><td>36/50</td><td>38/50</td><td>43/50</td><td>47/50</td><td>44/50</td><td>43/50</td><td>47/50</td><td>45/50</td></tr>
</table>
<u>Bodyweight and food consumption:</u>
No effects at any dose. No clinical signs of toxicity.

<u>Tissue specific findings:</u>
Liver was most sensitive target organ as evidenced by increased liver weight and histopathological findings. Histopathological findings also seen in the thyroid. There were some minimal haematological changes. Clinical chemistry parameters were not measured.

<u>Neoplastic findings</u> (includes all animals from terminal kill and that died during course of the study):

Liver:
<table>
<tr><td>Dose (ppm)</td><td>0</td><td>100</td><td>1000</td><td>6000</td><td>Historical control incidence (50 animals per control group)</td></tr>
<tr><td>Males: Hepatocellular adenoma</td><td>1</td><td>5</td><td>1</td><td>4</td><td>10 studies

Range: 0 – 4
Overall incidence: 7/500 (1.4%)</td></tr>
<tr><td>Males: Hepatocellular carcinoma</td><td>1</td><td>1</td><td>3</td><td>3</td><td>10 studies

Not observed</td></tr>
<tr><td>Females: Hepatocellular adenoma</td><td>1</td><td>0</td><td>1</td><td>0</td><td>10 studies

Range: 0 – 2
Overall incidence: 7/500 (0.8%)</td></tr>
<tr><td>Females: Hepatocellular carcinoma</td><td>0</td><td>0</td><td>0</td><td>1</td><td>10 studies

Not observed</td></tr>
</table>
[a] one animal with both adenoma and carcinoma
[b] two animals with both adenoma and carcinoma
</td>
</tr>
</table>

4.8.1 Non-human information

4.8.1.1 Carcinogenicity: oral

Carcinogenicity study in the rat

In a GLP and guideline-compliant reliable study, rats were administered penflufen in the diet for 104 weeks.

Details of non-neoplastic findings are provided in section 4.7 (repeated dose toxicity). There were no adverse treatment-related effects on survival. It is noted that survival in males at 104 weeks was below 50% in all groups including controls. The reduced survival in males is not considered treatment-related as mortality was comparable in all dose groups throughout the study. Survival was above 50% up to week 97 of the study; this was considered adequate for the assessment of carcinogenicity.

In treated females, there was a small increased incidence of benign tumours (hepatocellular adenoma and increased ovarian tubulostromal adenoma in some of the treatment groups. However, for both tumour types, the relationship to dose was unclear. In treated males, there was an increased incidence of malignant tumours (astrocytoma in the brain and increased histiocytic sarcoma of the haematopoietic system). These findings are considered further below.

Liver

In females, there was an increase in benign hepatocellular tumours (0%, 3%, 8% and 7% in the control, low, mid and high dose groups respectively). The incidences at the mid and high doses slightly exceeded the laboratory historical control range (0-5%) from 10 studies.

In males, the incidence of benign hepatocellular adenoma was within the historical control range and considered to be incidental.

In animals that died prematurely, hepatocellular adenoma occurred in one top dose male and one mid dose female. There was no increase in malignant liver tumours in either sex and no liver tumours occurred in the chronic phase of the study.

The main non-neoplastic liver findings are summarised in Table 18, below. Similar findings, together with liver enzyme induction, were also reported in the repeat dose toxicity studies with penflufen. Females were generally more sensitive than males. The treatment-related increased frequency of eosinophilic foci in females exceeded the historical control in incidence and severity in all dose groups, and may indicate pre-neoplastic changes with the potential to progress to tumours.

Given that the liver is clearly a target organ for penflufen, the small increase in benign tumours seen in females may have been treatment-related. However, there is no explanation for the absence of similar findings in males or the absence of malignant tumours and it is possible that the increased survival in the female-treatment groups (compared to control females) could have contributed to the increased frequencies seen.

Table 18: Summary of the main non-neoplastic findings in the liver in rats administered penflufen for 104 weeks

Parameter	Severity	Dietary concentration of Penflufen (ppm)							
		Males				Females			
		0	100	2000	7000	0	100	2000	7000
Number examined		60	60	60	60	60	60	60	60
Rel. liver wt. (% of body weight)		2.14	2.15	2.20	2.50** (+17%)	2.46	2.38	2.50	2.79** (+13%)
Liver: centrilobular to panlobular hepatocellular hypertrophy	Min	0	5	20	39	0	0	21	26
	Slight to mod	0	0	1	11	0	0	1	21
	Total	0	5*	21**	50**	0	0	22**	47**
Liver : hepatocellular brown pigment: focal	Min	0	1	8	21	0	0	13	19
	Slight	0	0	1	2	0	0	3	8
	Mod to marked	0	0	0	0	0	0	2	3
	Total	0	1	9**	23**	0	0	18**	30**
Liver: eosinophilic focus(i) of hepatocellular alteration	Min	22	26	26	20	25	24	23	23
	Slight	1	4	6	9	2	14	22	15
	Mod				1			1	1
	Total	23 (38%)	30 (50%)	32 (53%)	30 (50%)	27 (45%)	38 (63%)	46** (77%)	39* (65%)
Historical control[A] for eosinophilic foci in the liver		**Study range**		**Overall incidence**		**Study range**		**Overall incidence**	
	Min	4 – 41 (8 – 68%)		134/525 (26%)		0 – 30 (0 – 54%)		125/585 (21%)	
	Slight	0 – 6 (0 – 10%)		28/525 (5%)		2 – 8 (3 – 13%)		39/585 (7%)	
	Mod	0 – 2 (0 – 3%)		5/525 (1%)		0 -5 (0 – 8%)		9/585 (2%)	
	Total	2 – 47 (3 – 78%)		169/585 (29%)		5 – 33 (8 – 60%)		173/585 (30%)	

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

Brain

The incidence of astrocytoma in males marginally exceeded the maximum historical control incidence (3%) in the top dose group (1.7%, 0%, 0% and 5% in the control, low, mid and high dose groups respectively). The 3 males in the top dose group with astrocytoma all died prematurely during the study (the control male was found to have astrocytoma at the terminal kill).

The tumour frequency in males was only just outside the historical control range and no tumours were seen in females. No other obvious treatment-related changes in brain pathology were seen at necropsy. Metabolites of penflufen have been detected in the brain of exposed rats, but the brain is not a major target organ of exposure (see section on Toxicokinetics). Taking into account all of these factors, a clear indication of a carcinogenic response in the brain is considered to be lacking.

Haematopoietic system

In males, histiocytic sarcoma exceeded the maximum historical control incidence (3.3%) in all dose groups (0%,5%, 5%, and 8.3% in the control, low mid and high dose groups respectively). The 3

animals with tumours in the mid dose and 2/5 in the top dose died prematurely during the carcinogenicity phase of the study. There were no histiocytic sarcomas in the chronic phase (see section 4.7). According to the pathology report, these tumours originated in the haematopoietic tissues, although many affected rats also had metastasis. Treatment-related findings in the bone marrow, spleen, thymus and lymph nodes were not identified in the current study or in any of the other repeat dose studies.

There were no histiocytic tumours in treated females, although 3 control females were affected. It is not clear why treated males would be more susceptible to this tumour than treated females as there were only minor sex differences in tissue distribution and metabolism of penflufen in the metabolism studies. The available toxicokinetic information on penflufen suggests that females have a slightly higher level of systemic exposure than males (as measured by higher urinary excretion). Overall, although the very slight increase in histiocytic sarcoma frequency seen in treated male rats may have occurred by chance, the possibility of a very weak treatment-related effect cannot be excluded.

Ovary

There was an increased incidence of benign tubulostromal tumours in the top dose females that survived to the terminal kill (3.3%, 1.7%, 1.7% and 11.7% animals in the control, low, mid and high dose groups respectively). At the top dose, this exceeded the maximum historical control incidence of 6.7%. There were no lesions or tumours in the ovary in the chronic phase of the study and there was no treatment related increase in malignant tubulostromal tumours.

A slight increased incidence of ovarian tubulostromal hyperplasia was noted in the top dose group (5%, 6.7%, 1.7%, 11.7% at 0, 100, 2000 and 7000 mg/kg bw/ day respectively). However the severity of the lesion was not markedly increased, the incidence was within the historical control range, and none of the lesions were graded as more than 'moderate'. There was no change in ovary weights in any of the rat studies, and no other evidence indicative of a hormonal disturbance or any treatment-related effect in the ovaries.

Overall, there was an increased frequency of tubulostromal adenoma in the top dose animals and this exceed historical control levels. Evidence of target organ toxicity and other pre-neoplastic lesions is minimal, and no malignancy was seen, but the effect at the top dose may have been treatment-related.

Carcinogenicity study in the mouse

In a GLP and guideline compliant reliable study, mice were administered penflufen in the diet for 78 weeks. There were no adverse treatment-related effects on survival, and survival in all groups exceeded the minimal acceptable level. However, it is noted that there was increased survival in the males in the treated groups (60%, 76%, 86% and 94% survival in males in the controls, low, mid and high dose groups respectively).

Liver

The incidence of hepatocellular carcinoma exceeded the historical control incidence (0%) in all male dose groups, including the concurrent control (2%, 2%, 6% and 6% in the control, low, mid and high dose groups, respectively). Hepatocellular adenoma was increased in males in the low and top dose groups (1/50, 5/50, 1/50 and 4/50), but there was no dose response and the incidence at the top dose was within the historical control range (0-8%). All of these tumours were observed at terminal sacrifice with the exception of one male in the mid dose group which died during the course of the study.

Hepatocellular carcinoma was observed in 1/50 females in the high dose group, compared to 0 in all other groups and controls. There was no increase in adenomas in females.

Other findings in the liver are shown in Table 19, below. They included significant increased relative liver weights of 20% and 24% in males and females, respectively. Treated animals of both sexes had significant increases in diffuse centrilobular hepatocellular hypertrophy, with males affected in all dose groups, and females affected in the top and mid dose groups. Males in the top dose group had significantly increased incidence of diffuse hepatocellular vacuolation, and females in the top dose group had significantly increased incidence of diffuse hepatocellular macrovacuolation which was mainly periportal. However there was no evidence of pre-neoplastic changes such as foci of hepatocellular alteration.

Table 19: Summary of the main non-neoplastic and neoplastic findings in the liver in mice administered penflufen for 78 weeks

Parameter	Severity gr	Dietary concentration of Penflufen (ppm)							
		Males				Females			
		0	100	1000	6000	0	100	1000	6000
Number examined		48	49	49	48	50	50	50	50
Absolute liver weight. (g)		1.18	1.18	1.20	1.40** (+19%)	1.27	1.28	1.32	1.56** (+23%)
Rel. liver wt. (% of body weight)		4.48	4.39	4.57	5.39** (+20%)	5.32	5.40	5.56	6.60** (+24%)
Liver: diffuse centrilobular hepatocellular hypertrophy	Min	0	9	17	2	0	4	2	20
	Slight	0	3	9	15	0	1	1	10
	Moderate	0	1	3	29	0	0	0	1
	Total	0	13**	29**	46**	0	3	5*	31**
Liver : diffuse hepatocellular vacuolation	Min	8	10	7	13	16	7	14	12
	Slight	2	2	4	6	20	24	16	18
	Moderate	0	0	1	0	2	9	14	14
	Total	10	12	12	19*	38	40	44	44
Liver: diffuse hepatocellular macrovacuolation mainly periportal	Min	0	0	1	1	11	8	7	9
	Slight	0	0	0	0	3	3	0	22
	Moderate	0	0	1	0	2	9	14	14
	Total	0	0	1	1	14	11	7	41**
Liver: hepatocellular carcinoma		1	1	3	3	0	0	0	1
Liver: hepatocellular adenoma		1	5	1	4	1	0	1	0

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

As seen in the rat, the liver is clearly a target organ for penflufen. Given that hepatocellular carcinoma is extremely rare historically in the strain of mouse tested, the small numbers of tumours seen in both males and females administered penflufen may have been treatment-related. However, there was no dose-related increase in benign tumours and, as survival in males was higher in treated groups than in controls, the increased frequency of carcinoma seen in the mid and high dose groups could have been due to increased survival. Consequently the strength of supportive evidence is weak.

4.8.1.2 Carcinogenicity: inhalation

No relevant data available.

4.8.1.3 Carcinogenicity: dermal

No relevant data available.

4.8.2 Human information

No relevant data available.

4.8.3 Other relevant information

Mechanistic studies relevant to findings in the liver

Several non-guideline, non-GLP, 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 potential mode of action (MOA) is generally considered to be qualitatively not plausible for humans (see review by Elcombe *et al.* 2014). The studies assess CYP enzyme induction, cytotoxicity and replicative DNA synthesis in isolated rat and human hepatocytes, and enzyme induction and cell proliferation in rats and mice following 7 days administration of penflufen.

The studies are summarised below. Their relevance to the assessment of penflufen carcinogenicity is discussed in Section 4.9.4.

4.9.3.1 In vitro studies with rat hepatocytes

A study to investigate enzyme induction, cytotoxicity and cell proliferation was conducted in isolated female Wistar rat hepatocytes. Cells pooled from an unspecified number of animals were exposed for 3 days to penflufen (0.1, 1, 3, 10, 30, 100 µM; 95.6% pure), phenobarbital (10, 100, 1000 µM) or a solvent control (DMSO at a maximal concentration of 0.5% v/v). Phenobarbital is a model inducer of CAR/PXR and therefore was employed as a positive control. The investigation of cell proliferation also included cultures exposed to epidermal growth factor (EGF) (25 ng/ml) which served as a positive control for replicative DNA synthesis.

Table 20: Summary of in vitro studies with rat hepatocytes exposed to penflufen

Test system	Results and Conclusion	
Enzyme induction Enzyme activity (3 replicates/dose) measured using standard assay protocols for: PROD (CYP2B), BROD (CYP2B/CYP3A), BQ (CYP3A)	**Penflufen:** PROD (CYP2B activity): up to 5 fold ↑ versus control. BROD (CYP2B/CYP3A activity): up to 1.8 fold ↑ versus control. BQ (CYP3A activity): up to 2.4 fold ↑ versus control. **Phenobarbital:** ↑ enzyme activities in all three assays, at least 2 x effect seen with penflufen at a dose of 100 µM PROD ↑ 10 fold, BROD ↑ 5.7 fold, BQ ↑ 3 fold.	DAR 6.5.3 Unpublished Study (ref). (2011a) Non-guideline study GLP: No
Cytotoxicity Cell toxicity assay (6 replicates/dose) measured by ATP depletion assay kit	**Penflufen:** 11%↓ in ATP at 100 µM penflufen. **Phenobarbital:** No reduction in ATP in any other dose groups or in cells treated with phenobarbital.	
Study to investigate replicative DNA synthesis Replicative DNA synthesis (5 replicates/dose) measured by BrdU incorporation.	**Penflufen:** up to 1.7 fold ↑ compared to vehicle controls. **Phenobarbital:** up to 1.8 fold ↑ in DNA replication. **EGF:** 5.5 fold ↑ in DNA replication.	

Phenobarbital is known to be an inducer of the constitutive androstane receptor (CAR) and typically induces PROD, BROD activity, and to a lesser extent BQ. In this study, both penflufen and phenobarbital preferentially induced PROD of the CYP2B subfamily. With phenobarbital BROD (of the CYP2B/CYP3A superfamily) was induced to a greater degree than BQ (of the CYP3A superfamily), whereas penflufen induced BQ to a greater degree than BROD. The magnitude of enzyme induction by penflufen was less marked than seen with phenobarbital. Penflufen also caused a proliferative response in the rat hepatocytes that was of similar magnitude to that induced by

phenobarbital. Both substances appeared less potent at stimulating DNA synthesis than Epidermal Growth Factor (EGF), which was used as a positive control.

The ATP assay indicated that penflufen may be slightly cytotoxic but only at the highest dose tested (100 µM), whereas there was no evidence of cytotoxicity in phenobarbital (up to 1000 µM).

The proliferative response observed together with the profile of hepatic enzymes induced by penflufen, in particular the induction of PROD, suggests that penflufen may be an inducer of CAR/PXR in the female rat.

4.9.3.2 In vivo studies in rats

An *in vivo* study to investigate enzyme induction, cytotoxicity and cell proliferation was conducted in female Wistar rats. Groups of 5 rats were administered 0 or 7000 ppm (595 mg/kg penflufen; 99.6% purity) for 7 days. Another group received 7 daily doses of 80 mg/kg phenobarbital. The main findings are summarised in the following table.

Table 21: Summary of in vivo studies in rats exposed to penflufen

<table>
<tr><th>Test System</th><th>Results and Conclusion</th><th>Reference</th></tr>
<tr><td>Liver enzyme induction

Liver microsomes analysed for total cytochrome P450 using reduced CO differential spectrum via spectrophotometry.

Liver microsomes analysed for enzyme activity using standard assays for: EROD (CYP1A), PROD (CYP2B), BROD (CYP3A) and lauric acid hydroxylation (CYP4A), UDPGT-4Nitrophenol and UDPGT-Bilirubin..</td><td>7000 ppm penflufen (595 mg/kg bw/day)
61%↑ cytochrome P450 content
267% ↑ PROD
1568% ↑ BROD
172%↑ UDPGT-4Nitrophenol
277%↑ UDPGT-Bilirubin
No effect on CYP 4A

80 mg/kg bw/day phenobarbital
38%↑ cytochrome P450 content
810%↑ PROD
3789%↑ BROD
104%↑ UDPGT-4Nitrophenol
86%↑ UDPGT-Bilirubin
No effect on CYP 4A</td><td rowspan="2">Unpublished Study (ref). (2013)
Non-guideline study
GLP: No</td></tr>
<tr><td>Gene transcription

Cytoplasmic RNA isolated from pooled liver samples was analysed using quantitative PCR to measure gene transcription of Cyp 1A1, CYP2B1, CYP3A3, CYP4A1, UDPGTR2 (UDP glucuronosyltransferase), UGT1A6 (UDP glucuronosyltransferase), SULT2A2 (sulfotransferase), EPHX1 (epoxide hydrolase), GSTM4 (glutathione-S-transferase), P-450 oxydoreductase POR, and beta-microglobulin B2M.</td><td>Gene transcripts:
<table>
<tr><th>Gene transcripts</th><th>Phenobarbital 80 mg/kg bw/day</th><th>Penflufen 595 mg/kg bw/day</th></tr>
<tr><td>CYP1A1</td><td>No change</td><td>+411%</td></tr>
<tr><td>CYP2B1</td><td>+2747%</td><td>+554%</td></tr>
<tr><td>CYP3A3</td><td>+787%</td><td>+1049%</td></tr>
<tr><td>CYP4A1</td><td>No change</td><td>No change</td></tr>
<tr><td>UGTLA6</td><td>+220%</td><td>+174%</td></tr>
<tr><td>UDPGTR2</td><td>+167%</td><td>+181%</td></tr>
<tr><td>SULT2A2</td><td>-46%</td><td>+48%</td></tr>
<tr><td>EPHX1</td><td>+218%</td><td>+107%</td></tr>
<tr><td>GSTM4</td><td>+630%</td><td>No change</td></tr>
<tr><td>POR</td><td>-50%</td><td>-31%</td></tr>
</table></td></tr>
</table>

Cell proliferation Cell proliferation measurement using imunohistochemical staining of histopathology slides for BrdU of duodenum and liver sections. Nuclei stained using haematoxylin. BrdU was administered in the water. Other investigations conducted: gross pathology, liver and brain weight, histopathological exam of liver	**7000 ppm penflufen (595 mg/kg bw/day)** 10% ↓ body weight 17% ↑ relative liver weight No adverse liver histopathology ↑ cell proliferation in centrilobular and periportal area of liver (60%↑ - not statistically significant) **80 mg/kg bw/day phenobarbital** 12% ↑ absolute liver weight 14% ↑ relative liver weight Hepatocellular hypertrophy in 2/5 animals Hepatocellular single cell necrosis in 1/5 animals Increased hepatic mitoses in 1/5 animals ↑ cell proliferation in centrilobular area of liver (48%↑ - not statistically significant)	

Induction of Phase I liver enzymes was shown by significant increases in cytochrome P450 following treatment with penflufen or phenobarbital. Penflufen caused a marked increase in BROD and PROD that was similar to phenobarbital, although the magnitude was lower. Penflufen and phenobarbital also induced Phase II liver enzymes shown by increased UDP GT-4Nitrophenol and UDP GT-Bilirubin.

Penflufen also significantly increased Phase I and Phase II liver enzyme transcription. Like phenobarbital, penflufen treatment caused a marked increase in CYP 2B1(known to be influenced by CAR/PXR). Both substances also strongly upregulated CYP 3A3.

However, penflufen induced CYP 1A1 gene transcription. Although this may be under some influence of CAR, it is widely recognised as a marker of arylhydrocarbon receptor AhR induction. This receptor is involved in various cellular signalling pathways and dysregulation of these cellular processes may provoke a carcinogenic response. Phenobarbital had no effect on CYP 1A1 transcription or EROD activity.

Penflufen didn't produce an increase in glutathione-S-transferase (GST M4) gene transcription, which might have been expected of a substance that activates CAR/PXR. However, an increase was seen in mouse liver following dosing with penflufen (see below).

Liver enlargement and cell proliferation was evident in rats treated with penflufen or phenobarbital. However, adverse liver histology was only seen in rats treated with phenobarbital.
Overall, the findings in this study were generally consistent with activation of CAR/PXR. However, the induction of CYP 1A1 additionally implicates an inducing effect on AhR.

4.9.3.3 In vivo studies in mice

An *in vivo* study to investigate enzyme induction, cytotoxicity and cell proliferation was conducted in male C57BL/6J mice. Groups of 5 animals were administered 0 or 6000 ppm (1041 mg/kg

penflufen; 99.6% purity) for 7 days. Another group received 7 daily doses of 80 mg/kg phenobarbital. The main findings are summarised in the following table.

Table 22: Summary in vivo studies with mice exposed to penflufen

<table>
<tr><th>Test System</th><th>Results and Conclusion</th><th></th></tr>
<tr><td>Liver enzyme induction

Liver microsomes analysed for enzyme activity using standard EROD (CYP1A), PROD (CYP2B), BROD (CYP3A) and lauric acid hydroxylation (CYP4A), UDPGT-4Nitrophenol, UDPGT-Bilirubin assays.

Liver microsomes analysed for total Cytochrome P450 using reduced CO differential spectrum via spectrophotometry</td><td>6000 ppm penflufen (1041 mg/kg bw/day)

86%↑ cytochrome P450 content
66%↑ EROD
673% ↑ PROD
5679 ↑ BROD
57%↑ UDPGT-Bilirubin

80 mg/kg bw/day phenobarbital

99%↑ cytochrome P450 content
147%↑ EROD
2240%↑ PROD
16231%↑ BROD
102%↑ UDPGT-Bilirubin</td><td rowspan="3">Unpublished Study (ref). (2013a) Non-guideline study GLP: No</td></tr>
<tr><td>Gene transcription

Cytoplasmic RNA isolated from pooled liver samples was analysed using quantitative PCR to measure gene transcription of Cyp 1A1, CYP2B9, CYP2B10, CYP3A11, CYP4A1, UDP glucoronoyltransferases UGT1A1, UGT2B5, sulfotransferases SULT1A1, SULT2A2 and SULTN, Glutatione S-transferase GSTM4, Epoxide hydrolase EPHX1, P-450 oxydoreductase POR, and beta-microglobulin B2M.</td><td>Gene transcripts
<table><tr><th>Gene transcripts</th><th>Phenobarbital 80 mg/kg bw/day</th><th>Penflufen 1041 mg/kg bw/day</th></tr><tr><td>CYP 1A1</td><td>+69%</td><td>No change</td></tr><tr><td>CYP 2B9</td><td>+1614%</td><td>No change</td></tr><tr><td>CYP 2B10</td><td>+7113%</td><td>+1568%</td></tr><tr><td>CYP 3A11</td><td>+329%</td><td>+101%</td></tr><tr><td>CYP 4A10</td><td>No change</td><td>+35%</td></tr><tr><td>UGT 1A1</td><td>+230%</td><td>+57%</td></tr><tr><td>UGT 2B1</td><td>+141%</td><td>+42%</td></tr><tr><td>UGT 2B5</td><td>+89%</td><td>+47%</td></tr><tr><td>SULT 1A1</td><td>+105%</td><td>No change</td></tr><tr><td>SULT 2A2</td><td>+173%</td><td>No change</td></tr><tr><td>SULT N</td><td>+301%</td><td>+53%</td></tr><tr><td>EPHX 1</td><td>+125%</td><td>+52%</td></tr><tr><td>GST M4</td><td>+98%</td><td>+73%</td></tr><tr><td>P-450 POR</td><td>+203%</td><td>No change</td></tr></table></td></tr>
<tr><td>Cell proliferation

Cell proliferation measurement using imunohistochemical staining of histopathology slides for BrdU of duodenum and liver sections. Nuclei stained using haematoxylin.

BrdU was administered in the water</td><td>6000 ppm penflufen (1041 mg/kg bw/day)

11%/10% ↑absolute/ relative liver weight
↑Hepatocyte hypertrophy in 24/24 males versus 0 in controls

↑ cell proliferation in centrilobular and periportal areas of liver (approx. 60%↑ - not statistically significant)</td></tr>
</table>

Other investigations conducted: gross pathology, liver and brain weight, histopathological exam of liver	**80 mg/kg bw/day phenobarbital** ↑Hepatocyte hypertrophy in 25/25 males versus 0 in controls ↑ cell proliferation in centrilobular and periportal area of liver (approx. 3 fold↑ - not statistically significant)	

Penflufen caused significant phase I and phase II enzyme induction. Induction of Phase I liver enzymes was shown by significant increases in cytochrome P450 following treatment with penflufen; the magnitude of the effect was similar to that seen with phenobarbital. Penflufen caused a marked increase in EROD, PROD, BROD and UDPGT-bilirubin in a pattern that was similar to phenobarbital, although the magnitude of the effect less.

Penflufen significantly increased Phase I and Phase II liver enzyme transcription in a manner similar but not identical to phenobarbital, but the magnitude of the effect was about 50% lower. Both substances significantly increased CYP2B10, and to a lesser extent CYP 3A11, both of which are controlled by CAR/PXR. The main difference was that penflufen did not induce CYP2B9 compared to a 1614% induction by phenobarbital.

Penflufen caused an increase in liver weight and hepatocellular hypertrophy. Cell proliferation in the liver was also evident, although the magnitude of proliferation induced by penflufen was considerably lower compared to that seen with phenobarbital. The increase did not reach statistical significance as there was considerable variation between animals in the extent of proliferation recorded.

The pattern of enzyme activity, the liver hypertrophy and hepatocellular proliferation seen with penflufen are broadly indicative of CAR/PXR activation.

4.9.3.4 In vitro studies in human hepatocytes

A study to investigate the effects of penflufen on enzyme induction, cytotoxicity and cell proliferation was conducted in human hepatocytes.

Cryopreserved primary human female hepatocytes from one donor were cultured with 0, 0.1, 0.3, 1, 3, 10, 30 µM penflufen (95.6%), Additional cultures were exposed to phenobarbital (10, 100, 1000 µM) for comparative purposes. Epidermal growth factor (EGF) was employed as a positive control to demonstrate the inherent capacity of these cells to undertake replicative DNA synthesis.

Table 23: Summary of in vitro studies in human hepatocytes exposed to penflufen

Test system	Results and Conclusion	
Enzyme induction Enzyme activity (3 replicates/dose) measured after a 96 hour exposure period using standard assay protocols for: PROD (CYP2B) BROD (CYP2B/CYP3A) BQ (CYP3A).	**Penflufen:** PROD: no increase in activity. BROD: up to 1.45 fold ↑ versus control at 3 µM . BQ: up to 2 fold ↑ versus control at 30 µM . **Phenobarbital:** ↑ enzyme activities in all three assays at high doses (1000 µM) PROD ↑2.6 fold, BROD ↑ 5 fold, BQ ↑ 3.3 fold At lower doses (10 µM) phenobarbital had no effect.	DAR 6.5.3 Unpublished Study (ref). (2011b) Non-guideline study GLP: No
Cytotoxicity Cell toxicity assay (6 replicates/dose) measured by ATP depletion assay kit	**Penflufen**: 9% and 32% ↓ in ATP at 10 and 30µM penflufen respectively. **Phenobarbital**: 15%↓ in ATP at 1000 µM phenobarbital.	
Replicative DNA synthesis	**Penflufen:** No increase in replicative DNA synthesis. **Phenobarbital:** No increase in replicative DNA synthesis. **EGF** (25 ng/ml)**:** Marked (9-fold) increase in DNA replication.	

As indicated by the reduction in ATP levels, penflufen appeared to be slightly cytotoxic at the highest dose tested in this test system. Small increases in the activity of BROD and BQ were seen, but not in

the activity of PROD. The lack of PROD induction (a marker for CYP2B) by penflufen suggests it is not a potent inducer of CAR in human female hepatocytes. It did, however, produce modest increases in BROD and BQ. Whereas BROD is a marker both of CYP2B and CYP3A, increased BQ is a marker for CYP 3A1 induction, which is likely to be linked to PXR activation.

Penflufen exposure did not stimulate an increase in replicative DNA synthesis. In contrast, exposure to the positive control (EGF) produced a 9-fold increase in replicative DNA synthesis, indicating that the cultured cells could proliferate when exposed to appropriate stimuli.

Phenobarbital exposure also failed to stimulate proliferation of the hepatocytes. This substance was less toxic to the cells than penflufen and under the conditions of this study induced PRD as well as BROD and BQ.

Although this profile of responses was different to those seen with rat and mouse hepatocyte cultures, only one donor was used to source the hepatocytes and therefore care should be taken before reaching any firm conclusions about its relevance to the human population as a whole.

4.8.4 Summary and discussion of carcinogenicity

The carcinogenic potential of penflufen has been investigated in rats and mice. The various different tumour types that may have been induced by penflufen in these studies are considered below.

Liver tumours

There were small increases in the frequency of hepatocellular adenoma in male and female rats. Only the findings in females appeared to be biologically significant as the rate in males (2/60 at the top dose) was within the historical control range of the laboratory (0-5%) and only just above the concurrent control level (1/60). No adenomas were seen in concurrent control females, but there were 5/60 and 4/60 at the top 2 doses of penflufen. The historical control rate was 0-5%.

There were no clinical signs of toxicity in any dose group but penflufen caused adverse changes in the liver at the top two dose levels with females showing increased susceptibility to liver lesions compared to males, in particular increased eosinophilic foci provided evidence of pre-neoplastic changes in females. There was no evidence of a penflufen-mediated increase in the incidence of hepatocellular carcinoma in either male or female rats.

There was an increased incidence of liver carcinoma in male mice treated with penflufen in the top and mid dose groups that exceeded the concurrent and historical control incidence rates. An isolated case was seen in female mice at the highest dose level only. Small numbers of benign tumours were also evident in both males and females, but a clear dose-response was not established. Additionally, there were hypertrophic changes in the liver in mice with males being slightly more susceptible than females. However, there were no indications of pre-neoplastic changes in the liver, and no clinical signs of toxicity.

There are various possible mechanistic explanations that can be considered for this weak carcinogenic response in rats and mice. They are summarised in the following table.

Mode of action	**Data relating to penflufen**	**Conclusion**
Genotoxicity	Negative data in standard tests	Unlikely
Cytotoxicity	No evidence of a cytotoxic mode of action in the liver *in vivo* in either rats or mice. Slight cytotoxicity in rat hepatocytes *in vitro* (11% reduction in ATP at the highest dose tested).	Unlikely
PPARα receptor activation	No induction of CYP 4A1 gene transcription in rat or mice hepatocytes, and there was no evidence of peroxisome proliferation (a key marker of PPARα receptor activators) in histopathological examinations.	Unlikely
CAR/PXR receptor activation	Mechanistic studies show that penflufen induces changes in rats and mice consistent with this mechanism (see details below this table).	Plausible, but not definitive
AhR receptor activation	In female rats penflufen did not induce EROD activity, although a modest increase in CYP 1A1 transcription occurred. In male mice penflufen induced a slight increase in EROD activity but no CYP 1A1 gene transcription. These findings indicate that penflufen may be activating AhR. However the magnitude of these effects was considerably lower than the activation of PROD and BROD activity and CYP 2B and CYP 3A transcription all of which are associated with CAR and PXR activation.	Unlikely
Porphyria	In rats administered penflufen at high doses, there were foci of brown pigment in the liver. The cause of the brown pigment was not confirmed but it can be an indicator of iron accumulation. However peliosis and necrosis were absent and red blood cell parameters were normal.	Unlikely
Endocrine	There was a slight increase in tubulostromal hyperplasia and tubulostromal adenocarcinoma in female rats in the two year study, but no adverse effects were seen in the ovaries in any of the repeat dose studies of shorter duration to indicate any hormonal disturbances.	Unlikely
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 29/30 day immunotoxicity study.	Unlikely

In recognition that penflufen may be associated with a weak hepatocarcinogenic effect in rats and mice, the applicant sponsored a series of mechanistic studies (Section 4.9.3) to investigate a possible non-genotoxic mode of action involving liver stimulation via an axis of constitutive androstane receptor (CAR) and pregnane X receptor (PXR) induction. As discussed previously in detail by the Risk Assessment Committee, the key events in this process are considered to be:

- CAR activation
- Altered gene expression specific to CAR activation
- Increased cell proliferation
- Inhibition of apoptosis
- Clonal expansion leading to altered foci
- Liver adenomas/carcinomas

Such a non-genotoxic mode of action has been considered of limited relevance to humans (Elcombe *et al*, 3014).

The mechanistic studies showed that penflufen increased gene transcription and activity of Phase I and Phase II xenobiotic metabolising enzymes in the livers of rats and mice in a pattern that is broadly consistent with activation of CAR/PXR nuclear receptors. A similar induction profile was also seen in cultured human hepatocytes. In contrast, although penflufen clearly had the potential to induce hepatocellular proliferation in rats and mice, it did not induce proliferation in cultured human hepatocytes. Results such as these may indicate a lack of human relevance of the liver tumour findings seen in rats and mice.

Importantly, the strength of these mechanistic investigations is limited because cells from only one human donor were investigated. Although these cells responded as would have been expected to the control substances, a single donor is considered insufficient to represent the human population as a whole.

A critical assessment of the data is presented in the following table, with reference also to the results seen with the model substance, phenobarbital.

Key and associative events	**Evidence in rats and mice**	**Evidence in humans**
Activation of CAR	YES. In rats suggested through the *in vitro* and *in vivo* MOA studies with increased PROD and BROD and BQ activity and increased transcription of CYP2B and CYP 3A enzymes. Potency of penflufen was lower compared to phenobarbital. Similar findings seen in mice.	UNCLEAR. MOA study *in vitro* indicated increased BROD and BQ activity (with similar potency to rats). Potency of BROD and BQ induction was lower compared to phenobarbital. However, PROD activity (a key marker of CAR activation) was absent. Suggests penflufen may primarily induce PXR and not CAR in humans.
Altered gene expression	YES. In rats marked increase in CYP 2B1 and CYP 3A3 which are controlled by CAR/PXR. In mice marked increase in CYP 2B10 and CYP 3A11 which are controlled by CAR/PXR. Increased phase II liver enzyme transcription. However, Increased CYP 1A1 in rats indicates that other potential modes of action may be possible.	Uncertain Predicted to occur based on increased BROD and BQ activity. Insufficient information on possibility of CYP 1A1 induction.
Hypertrophy	YES. In rats, liver hypertrophy evident in both sexes. In mice, males were more susceptible to hepatocellular hypertrophy in the centrilobular region compared to females (hypertrophy in this region may be an indicator of enzyme induction).	Uncertain Not measured with penflufen but is predicted to occur in humans where CAR activation occurs based on published evidence in humans treated with anticonvulsant drugs.
Increased hepatocellular proliferation	YES. Significant proliferation *in vitro* with cultured female rat hepatocytes. Slight proliferation *in vivo* in female rats, moderate proliferation *in vivo* in male mice.	Inconclusive. Would be predicted not to occur based on published evidence for species specificity of CAR activators in the literature. However, the evidence to support the lack of a proliferative effect of penflufen in humans is limited to studies in hepatocytes from a single human donor and it is questionable whether this study in isolation is sufficiently robust to make a firm conclusion on the lack of relevance for humans.
Altered hepatic foci	YES. In female rats *in vivo.* None seen in male rats or in male or female mice *in vivo.*	No data

Liver tumours	YES, although the carcinogenic response was weak and its association to all the evidence for CAR/PXR activation not entirely convincing. Liver tumours increased in female rats and male mice. However, these sex-specific observations could not be explained by the mechanistic data (no studies conducted with male rats or female mice). The responses were very small and there was no increase in malignant tumours seen in rats. In rats, tumours were also seen in the ovary, brain and haematopoietic tissues. These tumours are not associated with CAR activation.	No data.

Inhibition of apoptosis and other associative events in the CAR associated tumour model have not been investigated (altered epigenetic changes, gap junctional intercellular communication and oxidative stress) however this is not considered to be a critical knowledge gap considering the other information available.

However, in conclusion, whilst this mode of action is considered to provide a plausible explanation for the slightly increased incidence of liver tumours seen in some groups of penflufen treated animals, a definitive conclusion is not possible on the basis of the available evidence.

Other tumours

In addition to the liver tumours, very small increased incidences of tumours in the ovary, haematopoietic system and brain were observed in rats administered penflufen. In contrast, only liver tumour incidence was increased in mice.

The histiocytic sarcomas, which were all considered to have originated in the haematopoietic system, occurred in males only. The incidences of this tumour type showed a slight dose-response and exceeded the historical control range. Treatment-related findings in the bone marrow, spleen, thymus and lymph nodes were not detected in any of the repeat dose studies in rats including the 2 year carcinogenicity study. Therefore there is no evidence to support a mode of action involving chronic injury in the haematopoietic system, and there is no evidence to support any alternative MOA. It is possible that these were incidental findings, but it's also plausible that they could indicate a very weak carcinogenic response to penflufen administration. Given that the aetiology of the tumours is unknown, their relevance to humans cannot be dismissed. The ovarian tubulostromal tumours were benign and only increased in the top dose group animals, where they marginally exceeded the historical control incidence. They were not accompanied by convincing evidence of a treatment-related increase in pre-neoplastic lesions in the ovary, so evidence for causality for the development of tubulostromal tumours is weak. As for the histiocytic sarcoma, the low incidence and lack of evidence for causality suggests these findings in the ovary were incidental, however a weak treatment-related effect cannot be excluded.

In males there was also a slight increase in malignant astrocytoma. This was most likely an incidental finding as it only exceeded historical control incidence by one animal, there were no pre-neoplastic lesions in the brain and the metabolism studies had shown that the brain had a relatively low level of exposure to penflufen compared to other tissues.

4.8.5 Comparison with criteria

As there is no evidence of penflufen carcinogenicity in humans, a category 1A classification would be inappropriate. Equally, as increased tumour incidences were seen in rats and mice that cannot be dismissed completely as being either incidental or of no relevance to humans, a position of no classification is not possible.

Given that increased rates of tumours were seen in both penflufen-treated rats and mice, a Category 1B classification could be considered. However, the following evidence indicates that this may not be appropriate:

- 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 possibility that a mode of action involving CAR activation was responsible for the slight increases in liver cancer has not been established unequivocally);
- The increased frequencies of non-hepatic tumours were only evident in rats;
- Some of the increases were of benign tumours only.

If penflufen did produce a biologically significant tumour response in rats and mice, this was very weak. A case could be made for no classification, on the basis of a lack of relevance to humans, However, as discussed above, relevance to humans cannot be dismissed for all the tumour types and the small increases above background levels make it difficult to conclude that they were incidental. Under these circumstances, the data appear to match the criteria for a Category 2 classification best.

There are no grounds to draw attention to a particular route of exposure on the label.

4.8.6 Conclusions on classification and labelling

Carc 2; H351 – Suspected of causing cancer

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, $p \leq 0.05$; ** significantly different from control, $p \leq 0.01$

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, $p \leq 0.05$; ** significantly different from control, $p \leq 0.01$

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

KE1 and KE2: CAR activation and altered gene expression

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.

4.9 Toxicity for reproduction

4.9.1 Effects on fertility

The effects of penflufen on reproductive performance and fertility have been investigated in a GLP and guideline-compliant multi-generation study in rats.

Table 24: Summary table of relevant reproductive toxicity studies – Fertility

Note: The LOAEL values are given for information only. They have been taken directly from documentation connected to the EFSA peer review of penflufen without further critical assessment.

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Rat: Wistar 30/sex/dose 2-generation reproductive study. Dietary administration. DAR B 6.6.1 Unpublished Study (ref). (2009) Penflufen purity 95.6% OECD 416 GLP: Yes	0, 200, 1000, 4000 ppm corresponding to 0, 12/15, 58/71, 252/293 mg/kg bw/day in m/f (based on lowest estimated dose levels from F0 and F1 parental animals)	**<u>Parental findings</u>** **200 ppm (12/15 mg/kg bw/day)** No adverse findings **1000 ppm (58/71 mg/kg bw/day)** F0 males: ↑ rel liver weight (7%) **4000 ppm (252/293 mg/kg bw/day)** F0 females: ↓ bodyweight (9%) F0 females: ↑ abs/rel liver weight (13%/21%) F0 females: ↑ hepatocellular hypertrophy (minimal) in 9/30 f versus 0 in controls F0 males: ↑ abs/rel liver weight (14%/20%) F0 males:↑ hepatocellular hypertrophy (minimal) in 11/30 m versus 0 in controls F1 females: ↑ abs/rel liver weight (9%/16%) F1 females: ↑ hepatocellular hypertrophy (minimal) in 3/30 f versus 0 in controls F1 males: ↓ bodyweight (8%) F1 males: ↑ abs/rel liver weight (14%/23%) F1 males: ↑ hepatocellular hypertrophy (minimal) in 11/30 m versus 0 in controls **<u>Reproductive findings</u>** **200 ppm (12/15 mg/kg bw/day)** No adverse findings **1000 ppm (58/71 mg/kg bw/day)** No adverse findings **4000 ppm (252/293 mg/kg bw/day)** F1:13% ↓ mean number of pups delivered F2:11% ↓ mean number of pups delivered

Dose (ppm)	0	200	1000	4000	Historical control range (for Wistar rats within 5 years of current study)
Number of litters F1	27	26	28	29	
Number of litters F2	27	26	30	30	
Mean litter size day 0 F1	10.6	10.1	10.6	9.2	17 Studies 9.8 - 12.8
Mean litter size day 0 F2	10.4	10.1	10.7	9.3	8 Studies 10.4 – 10.9
Birth index[1] (%) F1	94.2	92.4	94.1	90.1	
Birth index[1] (%) F2	96.7	91.7	95.7	91.9	
Live birth index (%) F1	99.7	99.7	99.7	98.4	
Live birth index (%) F2	100.0	100.0	100.0	100.0	
Viability index (%) F1	96.7	99.4	98.5	96.9	
Viability index (%) F2	98.7	98.7	100.0	100.0	
Lactation index (%) F1	95.8	100.0	99.1	100.0	
Lactation index (%) F2	99.5	99.2	99.6	99.2	

[1]No. of implantation sites per litter/no. of pups born per litter x 100

Offspring findings
200 ppm (12/15 mg/kg bw/day)
No adverse findings

1000 ppm (58/71 mg/kg bw/day)
No adverse findings

4000 ppm (252/293 mg/kg bw/day)
F1 and F2: approx. 10% ↓ in body weight
F1 and F2: ↓abs/rel spleen weight 12%/14%
Time to vaginal opening ↑12%/8% in F1/F2 pups

LOAEL for parents, offspring and reproductive parameters:
4000 ppm (252/293 mg/kg bw/day)

4.9.1.1 Non-human information

In a guideline-compliant GLP study, rats were administered penflufen in the diet starting with the F_0 generation at about 8-9 weeks old and continuing until F_2 generation animals reached puberty.

There were no treatment related deaths or clinical signs of toxicity. Signs of general toxicity in the F_0 and F_1 parents at 4000 ppm, the highest dose level tested, included reduced bodyweight gain of ca, 9% in F0 females and 8% in F1 males (marginal reduction in food consumption during pre-mating (6%) and lactation(7%)), along with increased liver weights and liver hypertrophy. At this dose, the mean litter size in both the F1 (9.2) and F2 (9.3) generations was slightly reduced compared to the concurrent control (10.6 and 10.4 in F1 and F2 respectively). This was only marginally below the laboratory historical control range (mean litter size of 9.8 - 12.8 in the F1 generation and 10.4-10.9 in the F2 generation, from 17 studies conducted within 5 years of the current study) and there were

no effects on other parameters. There were no treatment-related malformations or clinical signs of toxicity in the offspring. Treatment related findings were isolated to the top dose group in both generations and included a reduction in pup bodyweight (10%) during lactation and a reduction in spleen weight (12% abs and 14% rel). Preputial separation and vaginal opening were slightly delayed in both the F_1 and F_2 generations in the top dose group. These differences are considered to be secondary to the lower bodyweights.

4.9.1.2 Human information

No relevant data available.

4.9.2 Developmental toxicity

The developmental toxicity of penflufen has been investigated in rats and rabbits.

Table 25: Summary table of relevant reproductive toxicity studies – Development

Note: The LOAEL values are given for information only. They have been taken directly from documentation connected to the EFSA peer review without further critical assessment.

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Rat (Sprague-Dawley) 23/dose Developmental Oral gavage from GD 6 - 20 DAR B.6.6.2 Unpublished Study (ref). (2008) Penflufen purity 95.6% Vehicle methyl cellulose OECD 414 GLP: Yes	0, 30, 100, 300 mg/kg bw/day	**Maternal findings:** **300 mg/kg bw/day** 13% ↓ bw gain ↓ food consumption ↑ liver weight No adverse findings in any other dose group **Developmental findings:** No developmental toxicity observed up to the highest dose tested. *LOAEL* *300 mg/kg/day for maternal toxicity.* *No LOAEL determined for developmental toxicity.*
Rabbit (New Zealand White) 23/dose Developmental Oral gavage from GD 6 – 28 DAR B.6.6.3 Unpublished Study (ref). (2008) Penflufen purity 95.6%	0, 30, 100, 600 mg/kg bw/day	**Maternal findings:** **600 mg/kg bw/day** One animal killed on GD 25 due to severe loss of body weight. ↓ bw gain (26%) ↓ food consumption No adverse findings in any other dose group **Developmental findings:** Please refer to the table below

Vehicle methylcellulose

OECD 414
GLP: Yes

Parameter	Penflufen (mg/kg/day)			
	0	30	100	600
Number pregnant females	22	23	23	19
Post-implantation loss (%)	10.0	5.0	15.8	11.5
Total number of early resorptions (number per dam)	7 (0.3)	3 (0.1)	23 (1.1)	7 (0.4)
Total number of dead fetuses (% per litter)	12 (5.3%)	7 (3.1%)	12 (4.9 %)	16 (8.0%)
Total no. of fetuses examined	191	218	187	167
No. of malformed fetuses (litters)	3 (3)	7 (5)	5 (5)	2 (2)
Malformations	2 fetuses with various malformations of the ribs and vertebrae 1 fetus with multiple malformations; forelimb amelia, diaphragmatic hernia, absent forelimb bones	1 fetus with multiple malformations; gastroschisis, absent kidneys and skeletal (ribs, vertebrae, sternebrae, limbs) 3 fetuses with various skeletal malformations of the ribs and/or vertebrae and/or sternebrae 1 fetus with multiple malformations; gastroschisis, anasarca, short snout, malrotated forepaw and skeletal (stern brae). 1 fetus with absent right atrioventricular valve 1 fetus with diaphragmatic hernia	1 fetus with multiple malformations; micrognathia, cleft palate, short trunk, bent tail, malpositioned digits on forepaws and skeletal (small mandible, split/bent palatine/clavicle) 1 fetus with cardiovascular (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 fetus with hydropericardium 2 fetuses with skeletal (rib and vertebrae) malformations	1 fetus with multiple cardiovascular (dilated aortic arch and ascending aorta. Pulmonary trunk atresia. Small right ventricle, enlarged left ventricle) malformations 1 fetus with omphalocele

		<table><tr><td></td><td></td><td></td><td></td><td></td></tr></table> *LOAEL 600 mg/kg bw/day for maternal toxicity* *LOAEL not determined for developmental toxicity*

4.9.2.1 Non-human information

Rats
There were no maternal deaths or clinical signs of toxicity

There was no evidence of developmental toxicity in the rat up to the highest dose tested.

Rabbits
One female of the top dose was killed for human reasons on GD25 due to significant loss of body weight between GD 17 and 25. In surviving dams there were no clinical signs of toxicity but there was a reduction in mean maternal body weight between GD 6 and 8 compared to a maternal weight gain in controls. Overall, mean maternal bodyweight gain was reduced by 26% throughout the treatment period compared to controls. Food consumption was reduced by 18-27% on GD 6-22 but was comparable with controls thereafter.

There was an increase in early resorptions in the mid dose group, but as this was not observed in the top-dose group and there is no dose response it is not considered to be a treatment related finding. An increase in dead fetuses (which exceeded historical controls) was noted in the top dose group. However, this was largely attributable to a single female with 6 dead fetuses and considered to be incidental.

Malformations were reported in 3,7, 5 and 2 fetuses in the 0, 30, 100 and 600 mg/kg bw/day dose groups respectively. The findings were inconsistent, with a number of fetuses having multiple malformations with no relation to dose. Two fetuses from different litters in the low dose group were found to have gastroschisis (fissure of the abdominal wall), which exceeded the laboratory historical control range (HCD; 1 incidence from 10 studies conducted within 7 years of the current study). However, this was not seen in the mid or high dose groups in the current study and the affected fetuses had multiple malformations. The one incidence of omphalocele (a malformation of the abdominal wall at the umbilicus) in the high dose group also exceed the HCD (0 incidence from the same range). However, it is noted that this has been seen in 1 fetus from the low dose group and 2 fetuses from the mid-dose group in another comparable study (conducted by the same laboratory in 2003). The other findings were within the HCD of the laboratory. Overall, there is a lack of consistency between the findings observed in the different groups and these are therefore considered to be incidental; particularly as there was no corresponding increase in variations or in post implantation loss.

4.9.2.2 Human information

No relevant data available.

4.9.3 Other relevant information

No relevant data available.

4.9.4 Summary and discussion of reproductive toxicity

The reproductive toxicity of penflufen has been investigated in a guideline multi-generation study in the rat and in guideline developmental toxicity studies in rats and rabbits. The highest doses in each study were sufficient to induce some maternal toxicity.

In the multi-generation study there was no evidence that penflufen had a specific effect on fertility, sexual function or reproduction. In the high-dose group, slight reductions in the mean litter size in both generations and a 10% reduction in pup bodyweight in both sexes (accompanied by delays in vaginal opening that are considered to be secondary to the lower pup bodyweights) were likely to be secondary effects of the maternal toxicity (as indicated by reductions in body-weight gain and liver effects).

Penflufen did not result in any adverse effects on developmental toxicity in the rat. In rabbits, an increase in dead fetuses in the high-dose group (600 mg/kg/d) occurred together with maternal toxicity (reductions in body-weight gain, sacrifice of one female on humane grounds). A single incidence of omphalocele in the high-dose group was considered to be incidental. In conclusion, there was no evidence that penflufen had a specific effect on development.

4.9.5 Comparison with criteria

Category 1A (known human reproductive toxicant) is not appropriate as *there is no human evidence establishing a causal relationship* between exposure to penflufen and an adverse effect on fertility or development. Likewise, Category 1B is not appropriate as *there is no clear evidence* of an adverse effect on fertility or development in experimental animals.

Category 2 (suspected human reproductive toxicant) is also not appropriate because *there is no evidence of an adverse effect on fertility or development in experimental animals.* Slight reductions in the mean litter size and reductions in pup bodyweight were likely to be secondary effects of the maternal toxicity and are not considered to support classification. There were no adverse effects on development in the rat or rabbit. Therefore, it is proposed that the available data do not meet the criteria for classification.

4.9.6 Conclusions on classification and labelling

Not classified: Conclusive but not sufficient for classification

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.

Additional key elements

Range-finding study to the two-generation study

As RAC had unresolved questions regarding the choice of the top dose in the two-generation study (IIA 5.6.1/1), Industry provided details of a range-finding study to this two-generation study. In the range-finding study, 10 animals per sex per group were exposed to penflufen via diet throughout a 10-week premating phase, mating, and females further until weaning of their litters on PND 21. Litters were culled on PND 4 and all remaining F1 pups were sacrificed on PND 21. The main findings of this study, with the focus on litter size, are summarized below.

Dose (ppm)	0	100	2000	4000	7000
Dose (mg/kg bw/d) gestation	0	8.2	163	450	974
Dose (mg/kg bw/d) lactation	0	8.7	175	358	763
Maternal parameters					
Body weight GD 0 [g]	237	239	224	215*	219
Food consumption GD 0-20 [g/animal/day]	19.5	20.1	18.1	26.0	31.2*
Body weight PND 0 [g]	261	270	241	239	237
Reproductive parameters					
No. of dams with implants	10	10	7	9	7
Mean litter size PND 0	10.7	9.8	10.0	7.8	10.0
Mean no. of implantation sites	11.3	10.1	9.9	8.7	10.1
Post-implantation loss [%]	5.4	2.3	23.9[a]	13.8	1.6
Offspring parameters					
Pup body weight PND 0 [g]	5.8	6.2	6.3	6.0	5.6
Pup body weight PND 21 [g]	48.2	50.6	48.3	44.3	40.8**

* significantly different from control, $p \leq 0.05$; ** significantly different from control, $p \leq 0.01$

[a] the high value mainly due to one dam with only 2 implants and no pups

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	<u>Maternal findings</u> 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 <u>Developmental findings</u> The developmental findings are summarised in a separate table below

<u>Rat PNDT study</u>

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.

<u>Rabbit PNDT study</u>

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 required.

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).

Supplemental information - In depth analyses by RAC

Detailed information on maternal body weight and litter size in the 2-generation study (IIA 5.6.1/1)

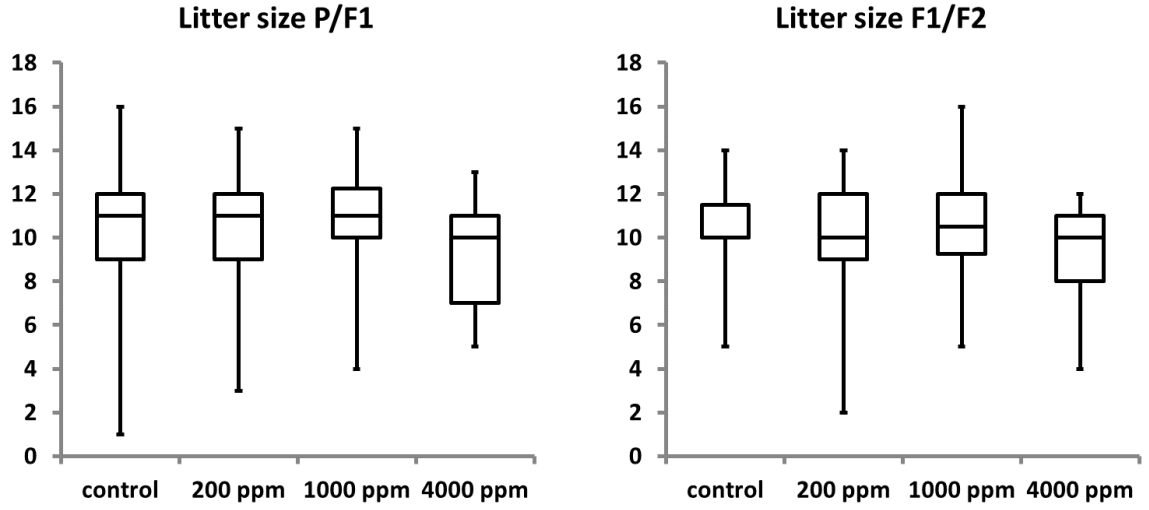
Maternal body weights at different time pointes are provided in the following table (data taken from the study report).

Maternal body weights in the two-generation study [g]				
	Dietary concentration of penflufen (ppm)			
	0	**200**	**1000**	**4000**
F0 generation				
Start of mating period	243	239	236	226** (–7%)
Gestational day 0	242	239	237	222** (–8%)
Gestational day 20	342	337	337	307** (–10%)
Postnatal day 0	269	265	259	242** (–10%)
Postnatal day 21	297	289	286	271** (–9%)
F1 generation				
Start of mating period	234	243	241	218** (–7%)

Gestational day 0	236	244	238	216** (–8%)
Gestational day 20	330	336	332	296** (–10%)
Postnatal day 0	257	263	258	233** (–9%)
Postnatal day 21	279	288	279	262** (–6%)

** significantly different from control, p ≤ 0.01

Distribution of litter sizes in both generations is shown graphically in the following two graphs (created by RAC from individual data). It is apparent that the reduction in top dose groups is a relatively consistent effect across the dams.



Legend: middle line = median; lower border = lower quartile; upper border = upper quartile; lower whisker = minimum; upper whisker = maximum

4.11 Aspiration Hazard

Not applicable as the substance is a solid.

4.12 Other Effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

The neurotoxicity of penflufen has been investigated in rats in an acute neurotoxicity study (summarised in section 4.2) and in a 90 day repeat-dose study, and a 90-day repeat dose neurotoxicity study (summarised in section 4.7). The studies are reliable, GLP and guideline compliant.

Acute neurotoxicity

It is concluded that there was no evidence of specific irreversible neurotoxicity in rats up to a maximum single oral dose of 2000 mg/kg bw.

Repeat dose neurotoxicity

There was no evidence of neurotoxicity in rats up to a maximum dose of 516/609 mg/kg bw/day in males/females. In the 90 day rat study up to a maximum dose of 949/1009 mg/kg bw/day in males/females (Steiblen 2006a) a neurotoxicity assessment was also conducted. There was no evidence of neurotoxicity in this study either.

4.12.1.2 Immunotoxicity

The immunotoxicity of penflufen has been investigated in rats in a GLP and guideline-compliant 29/30 day immunotoxicity study (summarised in section 4.7).

Spleen and thymus weights and the number of spleen cells per organ were not affected by treatment. There were no dose-related differences in the spleen cell immune response to an intravenous injection of sheep erythrocytes for a plaque forming assay. In conclusion there was no evidence of immunotoxicity in rats up to a maximum dose of 756/960 mg/kg bw/day in males/females.

4.12.1.3 Specific investigations: other studies

No data available.

4.12.1.4 Human information

No data available.

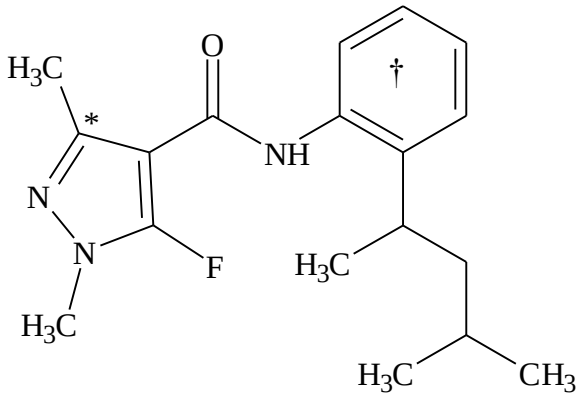
5 ENVIRONMENTAL HAZARD ASSESSMENT

Penflufen (referred to in test reports as BYF 14182) is an alkylamide fungicide initially intended for use as a potato tuber seed treatment for the control of 'black scurf'. Available environmental fate and hazard studies have been considered under EU Directive 91/414/EEC and summarised in the Draft Assessment Report, 2011 and subsequent DAR Addenda. The agreed endpoints from the peer review of penflufen under Directive 91/414/EEC are also included in the 2012 EFSA Conclusion (EFSA Journal 2012;10(8):2860)

Penflufen is currently under review as a biocide active substance and relevant information is also summarised in the Draft Competent Authority Report (dCAR 2016).

The key information pertinent to determining a classification is presented below. All radiolabelled studies used ^{14}C-penflufen with a purity of >99% as either (or both) [phenyl-UL-$^{13}C_6$/^{14}C] or [pyrazole-3-^{14}C] labels as shown in Figure 1.

Figure 1: Structure of penflufen indicating positions of the ^{14}C labels.



Positions of radiolabel:

† [phenyl-UL-^{14}C] penflufen

* [pyrazole-3-^{14}C] penflufen

The measured water solubility of penflufen in distilled water at 20 ºC is 12.4 mg/l at pH 6.5. With adjusted pH the water solubility was: 11.0 mg/l at pH4, 10.9 mg/L at pH 7 and 11.2 mg/l at pH 9.

Penflufen does not have any dissociation constants in the range of 1 < pKa <12.
All available data is based on penflufen as an isomer mixture.

Where available information on degradation products is included – full details of degradant names and structures are presented in Annex I.

5.1 Degradation

A summary of available valid information on the fate of penflufen is presented in Table 26 below.

Table 26: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aquatic hydrolysis OECD Guideline 111, GLP	Stable at pH 4,7 and 9 at 50 °C	Valid	DAR B.8.4.1 Unpublished Study (ref). (2008)
Aquatic photolysis EPA (Subdivision N, 161-2) and EU Council Directive 91/414/EEC, section 2, sub section 2.9.2, GLP	DT_{50} ≈ 130.6 days at 38.03°N (Athens, Greece) in June sunlight. DT_{50} ≈ 163.6 days at 51.3°N (London, UK) in July sunlight.	Valid	DAR B.8.4.2 Unpublished Study (ref). (2009)
Aquatic photolysis in natural water (River Rhine) EPA (Subdivision N, 161-2) and EU Council Directive 91/414/EEC, section 2, sub section 2.9.2, GLP	DT_{50} = 26.2 to 33.1 days at 38.03°N (Athens, Greece) in June sunlight. DT_{50} = 32.7 to 41.4 days at 51.3°N (London, UK) in July sunlight.	Valid	DAR B.8.4.2 Unpublished Study (ref). (2009)
UBA (Germany) guideline on Phototransformation and ECETOC polychromatic light source guideline, GLP	Quantum Yield: 0.0003737 DT_{50} = 210 to 293 days at 50°N (Germany) in spring/summer sunlight using GC-SOLAR model. DT_{50} = 210 to 270 days at 50°N (Germany) in spring/summer sunlight using Frank & Klöpffer model.	Valid	DAR B.8.4.2 Unpublished Study (ref). (2009)
Water/sediment simulation OECD Guideline 308, GLP	Dissipation DT_{50} whole system: 301 to 333 days Mineralisation: 0.8 to 10.7% AR at 120 days	Valid Aerobic system	DAR B.8.4.4 Unpublished Study (ref). (2008)
Water/sediment kinetic evaluation, FOCUS Working Group	Dissipation DT_{50} whole system geometric mean: 221 days	Calculation to single first order kinetics based on data from Sneikus (2008)	DAR B.8.4.4 Unpublished Study (ref). (2008)

5.1.1 Stability

Aqueous hydrolysis

An aqueous hydrolysis study (Koehn, 2008) is available following GLP and OECD Test Guideline 111. The study used phenyl-UL-$^{13}C_6/^{14}C$ radio labelled penflufen (1.0 mg a.s./l). Test solutions were incubated at 50 °C in at pH 4, 7 and 9 the dark for 7 days. No significant degradation was observed

and analysis showed ≥97.5% radioactivity as penflufen at study termination. On this basis, penflufen is considered hydrolytically stable.

Aqueous photolysis

Study 1

An aqueous photolysis study is available following GLP, US EPA Guideline Subdivision N, Series 161-2, EU Council Directive 91/414/EEC, section 2, sub section 2.9.2 and SETAC Procedures for assessing the environmental fates and ecotoxicity of pesticides. The study used [phenyl-UL-$^{13}C_6$/^{14}C] and [pyrazole-3-^{14}C] radio labelled penflufen (1.0 mg a.s./l). Test solutions were incubated at pH 7 for 137.5 experimental hours at 25 ^{o}C ± 1^{o}C under constant irradiation (wavelengths below 290 nm filtered out). Radiochemical balances were 100.2 to 106.5% AR.

A number of degradants were observed at low levels comprising a total of 22.2% AR. The maximum an individual degradant was observed was 4.8% AR (not identified). Mineralisation was low accounting for 1.1% AR at study termination.

Penflufen DT_{50} values at various latitudes were determined using single first order (SFO) kinetics and a single compartment model using MatLab with KinGUI. The study DT_{50} based on the mean of both labels was 17.3 experimental days. This equates to a DT_{50} at 38.03^{o}N (Athens, Greece) of 130.6 days in June and at 51.3^{o}N (London, UK) of 163.6 days in July.

Study 2

A second aqueous photolysis study is available following GLP, US EPA Guideline Subdivision N, Series 161-2, EU Council Directive 91/414/EEC, section 2, sub section 2.9.2 and SETAC Procedures for assessing the environmental fates and ecotoxicity of pesticides. The study used sterile natural river water from the Rhine.

Two radio labels (phenyl-UL-$^{13}C_6$/^{14}C] and [pyrazole-3-^{14}C]) were used with ^{14}C-phenyl radio labelled penflufen at 0.7 mg a.s./l. Test solutions were incubated for 70 experimental hours at 25 ^{o}C ± 1 ^{o}C under constant irradiation (wavelengths below 290 nm filtered out). The pH ranged from 8 to 8.98 for the pyrazole label and 7.98 to 9.14 for the phenyl label. Radiochemical balances were 96.6 to 103.7% AR and 105 to 106.1 % AR for the phenyl and pyrazole labels respectively.

Up to 15 degradants were observed at low levels with none ≥ 10% AR. Two degradants were observed with the pyrazole radio label: pyrazole-4-carboxamide penflufen and fluoro acid penflufen. Mineralisation was low accounting for 0.7% AR for the phenyl label and 0% AR for the pyrazole label at study termination.

Penflufen DT_{50} values at various latitudes were determined using single first order (SFO) kinetics and a single compartment model using MatLab with KinGUI. Considering the 2 labels, the DT_{50} at 38.03^{o}N (Athens, Greece) was 26.2 to 33.1 days in June and at 51.3^{o}N (London, UK), 32.7 to 41.4 days in July.

Study 3

A third investigation into photodegradation is available and considered to GLP. The quantum yield of penflufen was calculated using ECETOC methods to be 0.0003737. DT_{50} values were then estimated using 2 models:

- DT_{50} = 210 to 293 days at 50ºN (Germany) in spring/summer sunlight using GC-SOLAR model.
- DT_{50} = 210 to 270 days at 50ºN (Germany) in spring/summer sunlight using Frank & Klöpffer model.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not available.

5.1.2.2 Screening tests

Not available.

5.1.2.3 Simulation tests

A degradation in aerobic water-sediment system study is available following OECD Test Guideline 308 and GLP. The study used ^{14}C-penflufen with two labels: phenyl-UL-^{14}C] and [pyrazole-3-^{14}C]. Two German aerobic systems were used: 'Anglerweiher' and 'Hoenniger Weiher'. The water and sediment test conditions with a ratio of 3:1 are included in table 27 below. The systems were treated with approximately 52 µg penflufen per litre of water via the water surface.

Table 27: Water-sediment system test conditions

Criteria	Anglerweiher lake, Germany	Hoenniger Weiher pond, Germany
Water properties	pH: 7.4 Dissolved organic carbon: <2 ppm Oxygen: 106 % saturation Redox potential: 246 mV	pH: 6.8 Dissolved organic carbon: <2 ppm Oxygen: 106 % saturation Redox potential: 235 mV
Sediment properties	97% sand; 2% silt; 1% clay Organic carbon 0.3% pH: 7.0 Redox potential: unknown	57% sand; 38% silt; 5% clay Organic carbon 3.0% pH: 5.2 Redox potential: unknown

The study was conducted at 20 ºC, in the dark under aerobic conditions for up to 120 days.

Radioactivity was determined by Liquid Scintillation Counting (LSC) and subsequent analysis by High Performance Liquid Chromatography (HPLC) was undertaken. Total mean recoveries for both systems were >96% Applied Radioactivity (AR) for both labels at each sampling point.

Penflufen dissipated from the water phase to the sediment phase in both systems via partitioning with limited degradation in both phases.

Anglerweiher: In water penflufen decreased from initial 85.5/83.3% AR to 36.6/43.5% AR on day 120. In sediment penflufen increased from initial 8.9/7.4% AR to peak at 30.1% AR on day 7 for label 1 and 30.7% AR on day 30 for label 2.

Hoenniger Weiher: In water penflufen decreased from initial 90.6/92.3% AR to 10.6/6.7% AR on day 120. In sediment penflufen increased from initial 7.4/6.1% AR to peak at 76.7% AR and 77.8% AR on day 33 for both labels.

The degradation product penflufen-3-hydroxy-butyl (M01) was observed in both water and sediment. It reached a maximum in the Anglerweiher phenyl label system at day 120 of 10.7% AR in waters and 2.1% AR in sediment. It was considered that the degradant formed in sediment and subsequently partitioned into the water phase.

Whole system study dissipation DT_{50} values for both labels were as follows:
$DT_{50\ whole\ system}$: 333 days for Anglerweiher system following FOMC[1] kinetics
$DT_{50\ whole\ system}$: 301 days for Hoenniger Weiher system following DFOP[2] kinetics

Minimal mineralisation was observed with a maximum of 3.2% AR in Anglerweiher system and 1.1% AR in Hoenniger Weiher system after 120 days.

Additional statistical analysis (Sur, 2008) considered further kinetic assessment using the data from Sneikus, 2008. It considered a multi-compartment model for degradation following the FOCUS Work Group on degradation kinetics. Using Single First Order kinetics, $DT_{50\ whole\ system}$ values were as follows:

$DT_{50\ whole\ system}$: 170 to 183 days for Anglerweiher system
$DT_{50\ whole\ system}$: 259 to 295 days for Hoenniger Weiher system

The geometric mean value was 221 days.

5.1.2.4 Summary and discussion of degradation

Penflufen is considered hydrolytically stable.

Penflufen is susceptible to limited photodegradation. The experimental DT_{50} in sterile pure water was 130.9 days at 38.03°N in June sunlight and 163.6 days at 51.303°N in July sunlight. The actual degree of photodegradation in the aquatic environment depends on local conditions and seasons. Therefore, in reality the potential for aquatic photolysis is likely to be limited.

A ready biodegradation study is not available.

In an aerobic water-sediment study penflufen was observed to dissipate from the water column to sediment in two systems where adsorption and formation of 3-penfulyfen-3-hydroxy-butyl occurred before the latter partitioned between the water and sediment phases. Estimated study whole system DT_{50} values for penflufen were between 301 and 333 days. Minimal mineralisation was observed. Subsequent kinetic assessment derived a single first order geometric mean whole system DT_{50} of 221 days.

[1] First Order Multi Compartment

[2] Double First Order Parallel

Overall, the degradation information does not provide sufficient data to show penflufen is ultimately degraded within 28 days (equivalent to a half-life < 16 days) or transformed to non classifiable products. Consequently, penflufen is considered not rapidly degradable for the purpose of classification and labelling.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Following OECD Test Guideline 106 and GLP, a soil adsorption study is available investigating the adsorption of penflufen. The study used 5 soils from the UK and Germany and ^{14}C-penflufen. Soil pH in water ranged from 5.6 to 6.5 and organic carbon from 1.2 to 2.3%. Adsorption was not considered to be pH dependant although correlation between increasing organic carbon and adsorption was observed as expected. The Kf_{oc} values ranged between 209.6 and 409.5 ml/g. This equates to logKf_{oc} values between 2.32 and 2.61.

5.2.2 Volatilisation

Experimental data indicate the vapour pressure for penflufen is low at 4.1 x 10^{-7} Pa at 20 °C following OECD Test Guideline 104. The Henry's Law Constant (Bogdoll and Eyrich, 2009) was calculated at 20 °C and pH 7 to be 1.19 x 10^{-5} Pa m^3 mol^{-1} indicating penflufen is unlikely to partition from the water phase to air.

5.2.3 Distribution modelling

Not relevant for classification and labelling.

5.3 Aquatic Bioaccumulation

Table 28: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water (HPLC method)	Log K_{ow} 3.3 at pH 4, 20°C Log K_{ow} 3.3 at pH 7, 20°C Log K_{ow} 3.3 at pH 10, 20°C	Valid	Unpublished Study (ref). (2009)
Experimental aquatic BCF OECD Guideline 305, GLP	Penflufen steady state whole fish BCF: 16 l/kg wet weight Penflufen steady state whole fish BCF: 12 l/kg wet weight (normalised for 6% lipid content) Kinetic whole fish BCF: 100 to 103 l/kg based on Total Radioactivity Residues Depuration half-life DT_{50} whole fish: 0.439 to 0.527 days	Flow through, 28 days exposure, 14 days depuration Valid	DAR B.9.2.1.2 Unpublished Study (ref). (2009)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

No data available.

5.3.1.2 Measured bioaccumulation data

An experimental aquatic BCF study for penflufen (purity >99%) is available following GLP and OECD Guideline 305 (2009). The study used ^{14}C-penflufen (Phenyl-UL-$^{13}C_6/^{14}C$), a flow-through system with Bluegill Sunfish (*Lepomis macrochirus*) and two exposure concentrations; 0.45 and 4.5 µg/l with the aid of solvent dimethylformamide (DMF) at 0.1 ml/l. The exposure period ran for 28 days followed by a 14 day depuration period. Analysis of Total Radioactive Residues (TRR) was by HPLC (High Performance Liquid Chromatography) with radio detection. Analysis of parent and metabolites was by HPLC co-chromatography.

Penflufen was reached steady state by day 3 and was extensively metabolised. A rapid depuration half-life of 0.439 to 0.527 days was calculated and after 14 days 98% of the radioactivity was depurated.

The whole fish steady state BCF for penflufen was 16 l/kg wet weight. Lipid analysis was conducted on days 28 and 42. The penflufen lipid normalised (6% lipid content) whole fish steady state BCF was 12 l/kg wet weight.

Origin^{TM} non-linear kinetic computer modelling was employed to determine kinetic BCFs based on TRR. Whole fish kinetic BCFs based on Total Radioactive Residues (TRR) were 100 to 103 l/kg.

5.3.2 Summary and discussion of aquatic bioaccumulation

The experimental $\log K_{ow}$ for penflufen is 3.3 at pH 4, 7 and 10 and 20 ^{o}C.

Experimental kinetic whole fish BCFs are 100 to 103 l/kg based on TRR. The experimental whole fish steady state BCF for penflufen is 16 l/kg wet weight. The penflufen lipid normalised (6% lipid content) whole fish steady state BCF was 12 l/kg wet weight.

Overall, the $\log K_{ow}$ is considered to be below the CLP $\log K_{ow}$ trigger value of ≥ 4 and the whole fish BCF for parent penflufen (or TRR) is below the CLP trigger of ≥ 500 intended to identify substances with a potential to bioaccumulate.

5.4 Aquatic toxicity

A summary of available valid information on the aquatic toxicity of penflufen is presented in Table 29. A summary of valid information for degradants is also included in Annex II, Table 1.

Studies were reviewed under EU Directive 91/414/EEC and Regulation (EC) No. 528/2012 and considered valid. Further details are presented for studies conducted on the active substance penflufen but not for its degradants as these are less toxic and not considered further for classification of penflufen.

Table 29: Summary of relevant information on aquatic toxicity for penflufen (BYF 14182)

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg a.s./l)	
Acute toxicity to fish OECD Guideline 203, GLP, purity: 95.6%	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	0.31 (mm)	DAR B.9.2.1.1 Unpublished Study (ref). 2009a
Acute toxicity to fish OECD Guideline 203, GLP, purity: 95.6%	Bluegill Sunfish (*Lepomis macrochirus*)	Mortality	Static	96 hours	LC_{50}	0.45 (mm)	DAR B.9.2.1.1 Unpublished Study (ref). 2009b
Acute toxicity to fish OECD Guideline 203, GLP, purity: 95.6%	Fathead Minnow (*Pimephales promelas*)	Mortality	Static	96 hours	LC_{50}	0.116 (mm)	DAR B.9.2.1.1 Unpublished Study (ref). 2009
Acute toxicity to fish OECD Guideline 203, GLP, purity: 95.6%	Common carp (*Cyprinus carpio*)	Mortality	Static	96 hours	LC_{50}	**0.103 (mm)**	DAR B.9.2.1.1 Unpublished Study (ref). 2009
Acute toxicity to fish OECD Guideline 203, GLP, purity: 95.6%	Sheepshead Minnow (*Cyprinodon variegatus*)	Mortality	Static	96 hours	LC_{50}	1.15 (mm)	DAR B.9.2.1.1 Unpublished Study (ref). 2009c
Fish Early Life-Stage (FELS) toxicity OECD Guideline 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) for length** 0.0476 (mm) for survival, weight and morphological/ behavioural effects	DAR B.9.2.1.1 Unpublished Study (ref). 2009
Daphnia sp Acute Immobilisation OECD Guideline, 202 GLP, purity: 95.6%	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>4.66 (mm)	DAR B.9.2.1.3 Unpublished Study (ref). 2008

Acute toxicity OECD Guideline, 202 GLP, purity: 95.6%	Crayfish (*Procambarus clarkii*)	Acute	Static	96 hours	EC_{50}	>4.5 (mm)	DAR B.9.2.1.3 Unpublished Study (ref)., 2009a
Acute toxicity US EPA OPPTS 850.1025, GLP, purity:95.6%	Oyster (*Crassostrea virginica*)	Acute	Flow-through	96 hours	EC_{50}	1.3 (mm)	DAR B.9.2.1.3 Unpublished Study (ref). 2009
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)	DAR B.9.2.1.3 Unpublished Study (ref). 2008
Daphnia magna Reproduction OECD Guideline 211, GLP, purity: 95.6%	*Daphnia magna*	Survival; reproduction; growth	Semi-static	21 days	NOEC	1.53 (mm)	DAR B.9.2.1.3 Unpublished Study (ref). 2009d
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 95.6%	*Pseudo-kirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	>5.1 (mm) 0.52 (mm)	DAR B.9.2.1.4 Unpublished Study (ref). 2007
Lemna sp. Growth Inhibition Test OECD Guideline 221, GLP, purity: 95.6%	*Lemna gibba*	Growth	Semi-static	7 days	$ErC_{50(frond\ number)}$ $ErC_{50(dry\ weight)}$ $NOErC_{(frond\ number)}$ $NOErC_{(dry\ weight)}$	>4.7 (mm) >4.7 (mm) 2.4 (mm) ≥4.7 (mm)	DAR B.9.2.1.4 Unpublished Study (ref). 2009e

Notes:
mm refers to mean measured
*formerly *Selenastrum capricornutum*
Bold values indicate most sensitive acute and chronic endpoints

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Five acute toxicity to fish studies using penflufen (purity 95.6%) are available following GLP and OECD Test Guideline 203.

Study 1 (Unpublished Study (ref)., 2009a)

Using Rainbow Trout (*Oncorhynchus mykiss*) the nominal exposure range was 0.063, 0.125, 0.25, 0.5 and 1.0 mg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Study conditions were within the test guideline range and validation criteria were met. Analytical verification by Liquid Chromatography / Tandem Mass

spectrometry (LC/MS/MS) were 88 to 104% of nominal with measured concentrations 0.066, 0.110, 0.22, 0.51 and 0.93 mg a.s./l. The study 96-h LC_{50} was 0.31 mg a.s./l based on mean measured with 95% confidence interval 0.22 to 0.51 mg a.s./l. The study 96-h NOEC was 0.11 mg a.s./l based on mean measured.

Study 2 (Unpublished Study (ref)., 2009b)

Using Bluegill Sunfish (*Leopmis macrochirus*) the nominal exposure range was 0.063, 0.125, 0.25, 0.5 and 1.0 mg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Study conditions were within the test guideline range and validation criteria were met. Analytical verification by LC/MS/MS were 100 to 111% of nominal with measured concentrations 0.065, 0.129, 0.28, 0.51 and 1.0 mg a.s./l. The study 96-h LC_{50} was 0.45 mg a.s./l based on mean measured with 95% confidence interval 0.28 to 1.0 mg a.s./l. The study 96-h NOEC was 0.285 mg a.s./l based on mean measured.

Study 3 (Unpublished Study (ref)., 2009)

Using Fathead Minnow (*Pimephales promelas*) the nominal exposure range was 0.063, 0.125, 0.25, 0.5 and 1.0 mg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Study conditions were within the test guideline range and validation criteria were met. Analytical verification by LC/MS/MS were 85 to 92% of nominal with measured concentrations 0.055, 0.106, 0.22, 0.46 and 0.90 mg a.s./l. The study 96-h LC_{50} was 0.116 mg a.s./l based on mean measured with 95% confidence interval 0.055 to 0.22 mg a.s./l. The study 96-h NOEC was 0.055 mg a.s./l based on mean measured.

Study 4 (Unpublished Study (ref)., 2009)

Using Common Carp (*Cyprinus carpio*) the nominal exposure range was 0.0478, 0.0956, 0.191, 0.382 and 0.765 mg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Study conditions were within the test guideline range and validation criteria were met. Analytical verification by HPLC-UV were 98 to 128% of nominal with measured concentrations 0.061, 0.117, 0.196, 0.475 and 0.751 mg a.s./l. The study 96-h LC_{50} was 0.103 mg a.s./l based on mean measured with 95% confidence interval 0.083 to 0.128 mg a.s./l. The study 96-h NOEC was 0.061 mg a.s./l based on mean measured.

Study 5 (Unpublished Study (ref)., 2009c)

Using Sheepshead Minnow (*Cyprinodon variegatus*) the nominal exposure range was 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 mg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Study conditions were within the test guideline range and validation criteria were met. Analytical verification by HPLC-UV were 82 to 96% of nominal with measured concentrations 0.116, 0.21, 0.43, 0.82, 1.92 and 3.45 mg a.s./l. The study 96-h LC_{50} was 1.15 mg a.s./l based on mean measured with 95% confidence interval 0.82 to 1.92 mg a.s./l. The study 96-h NOEC was 0.43 mg a.s./l based on mean measured.

5.4.1.2 Long-term toxicity to fish

A 35-day flow-through chronic toxicity to fish study (Unpublished Study (ref)., 2009) using penflufen following GLP and OECD Test Guideline 210 is available. The study used Fathead Minnow (*Pimephales promelas*) and the following endpoints: time to hatch, hatching success,

survival and growth (length and dry weight). General observations were also recorded. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Study conditions were within the test guideline range and validation criteria were met. The nominal exposure range was 6.25, 12.5, 25, 50 and 100 µg a.s./l. Results were based on mean measured values: 6.21, 12.2, 23.4, 47.6 and 95.7 µg a.s./l. Validity criteria were met and the test is considered reliable. Significant effects were determined by ANOVA followed by the Dunnet's multiple means comparison test and the William's test if appropriate. The most sensitive endpoint was fish growth (length) where the 35-d NOEC was determined to be 23.4 µg a.s./l based on mean measured concentrations (equivalent to 0.0234 mg a.s./l).

The mean length at day 35 for solvent controls was 23.4 mm and when pooled with controls was 23.5 mm. The mean length at day 35 at treatment 47.6 µg a.s./l was 23.0 mm. Whilst statistically significant resulting in a NOEC of 23.4 µg a.s./l, this difference is minor and was noted in the pesticides risk assessment. The EFSA conclusion supported the NOEC of 23.4 µg a.s./l. For the purpose of classification, the NOEC is considered to lie within the range 10 to 100 µg a.s./l (equivalent to 0.01 to 0.1 mg/l).

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Study 1(Unpublished Study (ref). 2008)

A static acute toxicity to *Daphnia magna* study using penflufen is available following GLP and OECD Test Guideline 202. The nominal exposure range was 0.31, 0.63, 1.25, 2.5 and 5.0 mg a.s./l reflecting the limit of solubility in test media. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Results were based on mean measured values: 0.3, 0.61, 1.26, 2.33, and 4.66 mg a.s./l. Validity criteria were met and the test is considered reliable. Effects were observed at the highest exposure concentration with 40% immobilisation. The study 48-h LC_{50} was >4.66 mg a.s./l based on mean measured. The study 48-h NOEC was 2.33 mg a.s./l based on mean measured.

Study 2 (Unpublished Study (ref). 2009a)

A static acute toxicity to freshwater crayfish study is available using penflufen and *Procambarus clarkii*. The study was run to GLP and followed an adapted version of OECD Test Guideline 202. The nominal exposure range was 0.31, 0.63, 1.25, 2.5 and 5.0 mg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Results were based on mean measured values: 0.31, 0.64, 1.16, 2.3, and 4.5 mg a.s./l. Observations of sub-lethal effects and mortality were recorded. The study is considered valid and reliable. No mortality/ effects were seen at the highest test concentration and the study 96-h EC_{50} was >4.5 mg a.s./l based on mean measured. The study 96-h NOEC was 4.5 mg a.s./l based on mean measured.

Study 3 (Unpublished Study (ref). 2009)

A flow-through acute toxicity to the marine Eastern Oyster (*Crassostrea virginica*) is available using penflufen. The study was run to GLP and followed US EPA OPPTS 850.1025. The nominal exposure range was 0.31, 0.63, 1.25, 2.5 and 5.0 mg a.s./l. Exposure solutions were prepared with

the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Results were based on mean measured values: 0.23, 0.44, 0.9, 1.4, and 3.2 mg a.s./l. Mortality and shell deposition were recorded endpoints. The study is considered valid and reliable. Based on shell growth, the study 96-h EC_{50} was 1.3 mg a.s./l with 95% confidence interval 0.81 to 2.4 mg a.s./l based on mean measured. Statistically significant inhibition of shell growth was observed at all treatments except 0.44 mg/l where 11% inhibition was noted. This means the study 96-h NOEC was considered <0.23 mg a.s./l based on mean measured.

Study 4 (Unpublished Study (ref). 2008)

A flow-through acute toxicity to the marine Mysid *Americanmysis bahia* is available using penflufen. The study was run to GLP and followed US EPA OPPTS 850.1035. The nominal exposure range was 0.31, 0.63, 1.25, 2.5 and 5.0 mg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Results were based on mean measured values: 0.45, 0.76, 1.4, 2.6 and 4.7 mg a.s./l. The study is considered valid and reliable. Based on mortality the 96-h LC_{50} was 2.5 mg a.s./l with 95% confidence interval 1.4 to 4.7 mg a.s./l based on mean measured. The study 96-h NOEC was 1.4 mg a.s./l based on mean measured.

5.4.2.2 Long-term toxicity to aquatic invertebrates

A semi-static chronic toxicity to *Daphnia magna* study using penflufen is available following GLP and OECD Test Guideline 211. The study assessed the following endpoints: survival, reproduction, length and weight. The nominal exposure range was 0.094, 0.19, 0.38, 0.75 and 1.5 mg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Results were based on mean measured values: 0.10, 0.18, 0.37, 0.74 and 1.53 mg a.s./l. Validity criteria were met and the test is considered reliable. No significant effects were observed for any parameter. The study 21-d NOEC was 1.53 mg a.s./l based on mean measured reflecting the highest exposure concentration.

5.4.3 Algae and aquatic plants

Algae:

A static algal growth inhibition test using penflufen (purity 95.6%) and *Pseudokirchneriella subcapitata* is available following GLP and OECD Test Guideline 201 under static conditions. The nominal exposure range was 0.16, 0.31, 0.63, 1.25, 2.5 and
5.0 mg a.s./l. Exposure solutions were prepared with the aid of the solvent DMF at 0.1 ml/l and a solvent control was included. Results were based on mean measured values: 0.14, 0.28, 0.52, 0.99, 2.3 and 5.14 mg a.s./l. Validity criteria were met and the test is considered reliable. As 11% inhibition of growth was observed at the highest exposure concentration, the 72-h E_rC_{50} was >5.1 mg a.s./l based on mean measured concentrations. The 72-hour NOE_rC was 0.52 mg a.s./l based on mean measured concentrations.

Aquatic plants:

A semi-static 7-day toxicity to *Lemna gibba* study using penflufen is available following GLP and OECD Test Guideline 221. Exposure solutions were prepared with the aid of the solvent DMF (0.1ml/l) and a solvent control was included. The nominal exposure range was 0.32, 0.63, 1.25, 2.5 and 5.0 mg a.s./l. Results were based on mean measured values: 0.27, 0.54, 1.1, 2.4 and 4.7 mg

a.s./l. Validity criteria were met and the test is considered reliable. The study endpoints were frond number, frond yield, biomass, growth rate and fry weight. Based on 10% inhibition was observed at the highest exposure concentration, the study 7-d $E_rC_{50\ (frond\ number)}$ was >4.7 mg a.s./l based on mean measured. Similarly, 6.6% inhibition was observed at the highest exposure concentration for the growth rate (dry weight) endpoint, so the study 7-d $E_rC_{50\ (dry\ weight)}$ was also >4.7 mg a.s./l based on mean measured. The lowest growth rate 7-d NOE_rC was based on frond number at 2.4 mg a.s./l based on mean measured.

5.4.4 Other aquatic organisms (including sediment)

No valid data.

5.5 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

For the purpose of classification, penflufen is considered not rapidly degradable.

The experimental $logK_{ow}$ for penflufen is 3.3 at pH 4, 7 and 10 and 20 °C. Experimental kinetic whole fish BCFs are 100 to 103 l/kg based on TRR. The experimental whole fish steady state BCF for penflufen only is 16 l/kg wet weight. The penflufen lipid normalised (6% lipid content) whole fish steady state BCF was 12 l/kg wet weight. Overall, the $logK_{ow}$ is considered to be below the CLP $logK_{ow}$ trigger value of ≥ 4 and the whole fish BCF for parent penflufen (or TRR) is below the CLP trigger of ≥ 500 intended to identify substances with a potential to bioaccumulate.

Identified degradants are relatively less toxic than the parent substance (see Annex II) and are not considered further for classification of penflufen.

Aquatic acute toxicity data on penflufen are available for fish, invertebrates, algae and aquatic plants. Fish are the most acutely sensitive trophic group with Common Carp (*Cyprinus carpio*) marginally the most sensitive followed by Fathead Minnow (*Pimephales promelas*). The lowest acute value is a 96-hour LC_{50} is 0.103 mg a.s./l. On this basis penflufen should be classified as Aquatic Acute 1 with an M factor of 1.

Adequate chronic toxicity data on penflufen are available for fish, invertebrates, algae and aquatic plants. The lowest value is a 35-day NOEC for Fathead Minnow (*Pimephales promelas*) of 0.0234 mg a.s./l. Given this is 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.

5.6 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Aquatic Acute 1; H400: Very toxic to aquatic life

Acute M factor = 1

Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Chronic M factor = 1

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} ≤ 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 ≤ 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 $E_rC_{50\ (\text{frond number})}$ 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 $E_rC_{50\ (\text{dry weight})}$ 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.**

5.7 Hazardous to the ozone layer

5.7.1 Short summary and overall relevance of the provided information on ozone layer hazard

Not applicable as penflufen is not mentioned as a controlled substance in the Annexes to the Montréal Protocol. Furthermore, it is not expected to enter in contact with stratospheric ozone molecules given its physico-chemical parameters and molecular structure.

5.7.2 Comparison with the CLP criteria

Not applicable as penflufen is not mentioned as a controlled substance in the Annexes to the Montréal Protocol.

5.7.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not classified – Conclusive but not sufficient for classification

6 OTHER INFORMATION

No other relevant information.

7 REFERENCES

- Draft Assessment Report (DAR) – Penflufen - Volume 3, Annex B.2: Physical and chemical properties – August 2011
- Draft Assessment Report (DAR) – Penflufen - Volume 3, Annex B.1 – August 2011
- Draft Assessment Report (DAR) – Penflufen - Volume 3, Annex B.6: Toxicology and Metabolism – August 2011
- Draft Assessment Report (DAR) – Penflufen - Volume 3, Annex B.8: Environmental Fate and Behaviour – August 2011
- Draft Assessment Report (DAR) – Penflufen - Volume 3, Annex B.9: Ecotoxicology – August 2011
- Draft Assessment Report (DAR) – Penflufen - Volume 4, Annex C – Confidential Information
- Addenda to Draft Assessment Report – 2012
- EFSA Journal 2012;10(8):2860
- Competent Authority Report – CAR – Penflufen – PT8 – 2016
- Elcombe, CR., Peffer, R.C., Wolf, D.C., Bailey, J., Bars, R., Bell, D., Cattley, R.C., Ferguson, S.S, Geter, D., Goetz, A, Goodman, J.I., Hester, S., Jacobs, A., Omiecinski, C.J., Schoeny, R., Xie, W and Lake, B.G. 2014 Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with Phenobarbital as a model constitutive androstane receptor (CAR) activator. Critical Reviews in Toxicology, 44, 64-82

A full reference list for the unpublished studies is provided as a confidential Annex (Annex III) to the CLH report.

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.

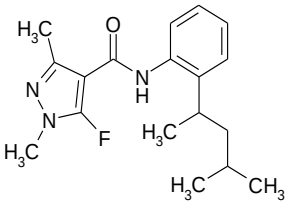
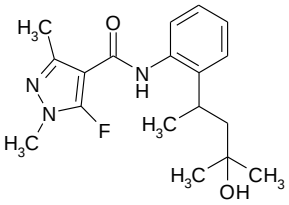
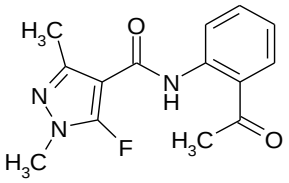
8. ANNEXES

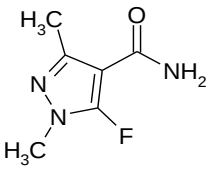
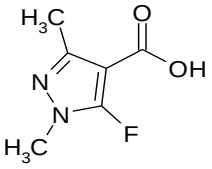
ANNEX I – Parent and degradant information: code, chemical name and structure.

ANNEX II - Aquatic toxicity data for penflufen degradants.

ANNEX III – CONFIDENTIAL – Full References

ANNEX I – Parent and degradant information: code, chemical name and structure.

	Report name, Structure IUPAC name CAS name, [CAS number]	**Molecular formula molar mass Other names / codes**
a.s.	**Penflufen (parent substance)**  N-[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl-1*H*-pyrazole-4-carboxamide (IUPAC) 1*H*-Pyrazole-4-carboxamide, N-[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl- (CAS) CAS-No.: 494793-67-8	C_{18} H_{24} F N_3 O 317.41 g/mole AE 1698405 BYF 14182
M01 Soil and aquatic degradant	**BYF 14182-3-hydroxy-butyl**  5-fluoro-N-[2-(3-hydroxy-1,3-dimethylbutyl)phenyl]-1,3-dimethyl-1*H*-pyrazole-4-carboxamide (IUPAC)	C_{18} H_{24} F N_3 O_2 333.41 g/mol BCS-AA10006
M02 Soil degradant	**BYF 14182-pyrazolyl-AAP**  N-(2-acetylphenyl)-5-fluoro-1,3-dimethyl-1*H*-pyrazole-4-carboxamide (IUPAC)	C_{14} H_{14} F N_3 O_2 275.28 g/mol AE 2300037 BCS-AF73126

	Report name, Structure IUPAC name CAS name, [CAS number]	**Molecular formula molar mass Other names / codes**
M58 Aquatic degradant	**BYF 14182-pyrazole-4-carboxamide**  5-fluoro-1,3-dimethyl-1*H*-pyrazole-4-carboxamide	C_6 H_8 F N_3 O 157.15 g/mol BCS-AA10791 ELB13168 SES10133-1-1
M60	**BYF 14182-fluoro acid**  5-fluoro-1,3-dimethyl-1*H*-pyrazole-4-carboxylic acid	C_6 H_7 F N_2 O_2 158.13 g/mol AE 1898258 ELB 10856

ANNEX II – Aquatic toxicity data for penflufen degradants.

Table 1: Summary of relevant information on aquatic toxicity for penflufen degradants

Degradant / Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/l)	
Penflufen-3-hydroxy-butyl (M01)							
Acute toxicity to fish OECD Guideline 203, GLP, purity 99.4%	Common Carp (*Cyprinus carpio*)	Mortality	Static	96 hours	LC_{50}	>15.7 (mm) limit test	DAR B.9.2.1.5.1 Unpublished Study (ref)., 2009a
Daphnia sp Acute Immobilisation OECD Guideline, 202, GLP, purity 99.4%	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>62 (mm) limit test	DAR B.9.2.1.5.2 Unpublished Study (ref). 2009f
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 99.4%	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	>75 (n) 18 (n) Supported by analytical verification	DAR B.9.2.1.5.3 Unpublished Study (ref). 2009b
Penflufen-pyrazolyl-AAP (M02)							
Acute toxicity to fish OECD Guideline 203, GLP, purity 99.6%	Common Carp (*Cyprinus carpio*)	Mortality	Static	96 hours	LC_{50}	>0.799 (mm) limit test	DAR B.9.1.1.5.1 Unpublished Study (ref)., 2009b
Daphnia sp Acute Immobilisation OECD Guideline, 202, GLP, purity 99.6%	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	>3.12 (mm) limit test	DAR B.9.2.1.5.2 Unpublished Study (ref). 2009c
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 99.6%	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	>1(n) 0.977 (n) Supported by analytical verification	DAR B.9.2.1.5.3 Unpublished Study (ref). 2009d

Notes:
mm refers to mean measured concentrations
n refers to nominal concentrations
*formerly *Selenastrum capricornutum*

ANNEX III

See confidential attachment.