Committee for Risk Assessment

RAC

Annex 1

Background document

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

Pencycuron (ISO);
1-[(4-chlorophenyl)methyl]-1-cyclopentyl-3-phenylurea

EC number: 266-096-3
CAS number: 66063-05-6

CLH-O-0000001412-86-32/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

04 December 2014

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Pencycuron

EC Number: 266-096-3

CAS Number: 66063-05-6

Index Number:

Contact details for dossier submitter: **Bureau REACH,**

RIVM,

The Netherlands,

bureau-reach@ rivm.nl

Version number: **4** **Date:** **April 2014**

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	*1-(4-chlorobenzyl)-1-cyclopentyl-3-phenylurea*
EC number:	*266-096-3*
CAS number:	*66063-05-6*
Annex VI Index number:	*Substance not listed in Annex VI*
Degree of purity:	*≥ 980 g/kg (technical material)*
Impurities:	*There are no impurities of toxicological, environmental and/or other significance in the active substance as manufactured.*

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	No entry listed in Annex VI, CLP.
Current proposal for consideration by RAC	Aquatic chronic 1 (H410) Chronic M-factor of 1.
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic chronic 1 (H410) Chronic M-factor of 1.

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

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	none	Not classified	Conclusive but not sufficient for classification.
2.2.	Flammable gases	Not classified	None	Not classified	Conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	None	Not classified	Conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	None	Not classified	Conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	None	Not classified	Conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	None	Not classified	Conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	None	Not classified	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	None	Not classified	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	None	Not classified	Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	None	Not classified	Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	None	Not classified	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	None	Not classified	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	None	Not classified	Conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	None	Not classified	Conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	None	Not classified	Conclusive but not sufficient for classification

2.16.	Substance and mixtures corrosive to metals	Not classified	None	Not classified	Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Not classified	None	Not classified	Conclusive but not sufficient for classification
	Acute toxicity - dermal	Not classified	None	Not classified	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	None	Not classified	Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	None	Not classified	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	None	Not classified	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	None	Not classified	No data
3.4.	Skin sensitisation	Not classified	None	Not classified	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	None	Not classified	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	None	Not classified	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	None	Not classified	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	Not classified	None	Not classified	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	None	Not classified	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	None	Not classified	Conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic chronic cat 1 (H410)	Chronic M-factor 1	Classified	
5.1.	Hazardous to the ozone layer	Not classified	none	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: Signal word: Warning
Hazard statements: H410: very toxic to aquatic life with long lasting effects

Precautionary statements:	Not proposed as P-statements are not included in Annex VI of CLP

Proposed notes assigned to an entry:

A note is not proposed.

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Pencycuron is a plant protection product which has been included in Annex I of Directive 91/414/EEC. The classification has not been assessed before by RAC or TC-C&L and the substance is not included in Annex VI of CLP. Therefore, RAC is requested to review all hazard classes including those hazard classes for which no classification is proposed.

The 2nd ATP has implemented the 3rd revised edition of GHS in which classification and assignment of M-factors can also be based on chronic aquatic toxicity. Based on the criteria of the 2nd ATP, a classification and an M-factor based on the chronic aquatic toxicity is proposed.

Pencycuron has been assessed in the Draft Assessment Report (2006), the addendum and resubmission of the Draft Assessment Report (2009) of The Netherlands prepared in the context of the possible inclusion of pencycuron in Annex I of Council Directive 91/414/EEC (DAR 2006 + 2009 and subsequent addenda, RMS The Netherlands) concerning the placing of plant protection products on the market. The DAR and the addenda are available at the EFSA website (http://www.efsa.europa.eu/ ; search: “Pencycuron”; DAR and addenda within the background documents). All references should be red as “as summarised in the DAR” as the original studies were not used for this CLH proposal.

The conclusions on the peer review of pesticide risk assessment of pencycuron were published in the EFSA Journal (8 (10): 1828, 2010). EFSA proposed the following classification with regard to ecotoxicological data R52/R53 “Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment”.

The DAR (resubmitted, 2009) is available via: http://dar.efsa.europa.eu/dar-web/provision.

There is currently no REACH registration of pencycuron (ECHA registration database as consulted on 27 June 2012).

2.2 Short summary of the scientific justification for the CLH proposal

In the current CLH report, a harmonised classification and labelling for Aquatic chronic toxicity with M-factors for pencycuron are proposed. Pencycuron is not rapidly degradable. It is proposed to classify pencycuron as Aquatic Chronic cat.1 (H410) based on a NOEC value of 0.05 mg/L for Daphnia magna. A harmonised M-factor of 1 for chronic toxicity in accordance with the 2nd ATP criteria is proposed.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation.

No harmonised classification exists for pencycuron in Annex VI, table 3.1.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

2.4 Current self-classification and labelling

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

The following classification and labelling proposals are reported in the CLP-inventory: Aquatic Chronic 4 (H413) submitted by 23 notifiers and Aquatic Chronic 1 (H410) submitted by 2 notifierrs.

2.4.2 Current self-classification and labelling based on DSD criteria

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Pencycuron is an active substance in the meaning of Directive 91/414/EEC as replaced by Regulation (EC) No 1107/2009 and therefore subject to harmonised classification and labelling (Regulation EC no 1272/2008, article 36.2).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

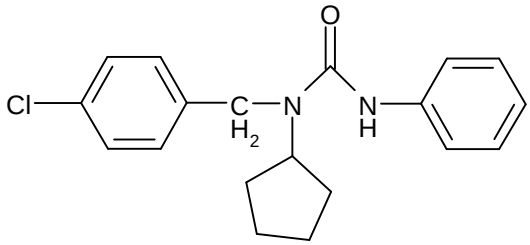
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	266-096-3
EC name:	1-[(4-chlorophenyl)methyl]-1-cyclopentyl-3-phenylurea
CAS number (EC inventory):	
CAS number:	66063-05-6
CAS name:	N-[(4-chlorophenyl)methyl]-N-cyclopentyl-N'-phenylurea
IUPAC name:	1-(4-chlorobenzyl)-1-cyclopentyl-3-phenylurea
Iso-name	Pencycuron
CLP Annex VI Index number:	-
Molecular formula:	$C_{19}H_{21}ClN_2O$
Molecular weight range:	328.8

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Pencycuron	≥ 980 g/kg		

Current Annex VI entry:

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
			All impurities have been claimed confidential and therefore provided in IUCLID. *There are no impurities of toxicological, environmental and/or other significance in the active substance as manufactured.*

Current Annex VI entry:

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
				All additives have been claimed confidential.

Current Annex VI entry:-

1.2.1 Composition of test material

The studies were performed with technical pencycuron with a purity range of 97.6% to 99.4%. This represents the above mentioned compostition of pencycuron (≥ 980 g/kg). No information regarding the impurities in the batches used for testing is available.

1.3 Physico-chemical properties

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	Colourless, white crystalline powder	DAR, vol 3.B2.1 (2009)	Visual observation
Melting/freezing point	128 °C; 132 °C	DAR, vol 3.B2.1 (2009). EFSA Journal (8 (10): 1828, 2010)	EEC A.1; atmospheric pressure. DSC method (pure 98.7%) Active substance after re-crystallisation (99.5%)
Boiling point	283 – 289 °C 283 – 288 °C	DAR, vol 3.B2.1 (2009) EFSA Journal (8 (10): 1828, 2010)	EEC A.2; atmospheric pressure. DSC method. Boiling occurs under decomposition.. (pure 98.7%)
Relative density	D^{20}_{4}: 1.24 1.22 g/cm^3 at 20 °C (99.5%)	DAR, vol 3.B2.1 (2009) EFSA Journal (8 (10): 1828, 2010)	EEC A.3 gas comparison method (pure 98.7%)
Vapour pressure	$4x10^{-7}$ Pa at 20° C $9.3x10^{-7}$ Pa at 25° C 5.10^{-10} Pa at 20° C 2.10^{-9} Pa at 25 °C	DAR, vol 3.B2.1 (2009) EFSA Journal (8 (10): 1828, 2010)	EEC A.4. Extrapolated values. Vapour pressure balance. (pure 98.7%). Extrapolated values (purity: 99.5%). Lower value is more reliable, due to higher purity of the substance.
Surface tension	Substance is not surface active 72.1 mN/m at 20 °C	DAR, vol 3.B2.1 (2009) EFSA Journal (8 (10): 1828, 2010)	According to EEC A.5: not applicable, because the water solubility is < 1 mg/L. A 90% saturated aqueous solution.
Water solubility	At 20 °C: 0.35 mg/L at pH 4.0 0.26 mg/L at pH 6.7 (not buffered) 0.29 mg/L at pH 9.0 0.3 mg/L at 20 °C	DAR vol 3, B 2.1 (2009) EFSA Journal (8 (10): 1828, 2010)	EEC A.6, column-elution method (pure 98.7%). No influence of pH.
Partition coefficient n-octanol/water	Log Kow: 4.0 at 25 °C 4.7 at 20 °C	DAR, vol 3.B2.1 (2009)	HPLC-shake flask method (pure 98.7% - 99.8%)
Flash point	Not applicable		
Flammability	Not highly flammable	DAR, vol 3.B2.1 (2009))	EEC A.10 (pure 99.6%)
Explosive properties	Not explosive	DAR, vol 3.B2.1 (2009))	EEC A. 14 (pure 99.6%)
Self-ignition temperature	Not auto flammable	DAR, vol 3.B2.1 (2009))	EEC A.16 (pure 99.6%)

Oxidising properties	Not oxidising	DAR, vol 3.B2.1 (2009)	EEC A.17 (pure 98.7%)
Granulometry	Data not available		
Stability in organic solvents and identity of relevant degradation products	Data not available		
Dissociation constant	Substance has neither acidic nor basic properties in aquatic solutions	DAR, vol 3.B2.1 (2009)	OECD 112. Spectrophotometric method (pure 98.7%)
Viscosity	Not applicable		
Volatility, Henry's law constant	5.10^{-7} $Pa.m^3.mol^{-1}$ at 20 °C 5.10^{-4} $Pa.m^3.mol^{-1}$ at 20 °C	DAR vol 3, B2.1 (2009) EFSA Journal (8 (10): 1828, 2010)	Calculated value. As the lower vapour pressure is more reliable, the lower Henry's law constant is also more reliable.

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

Pencycuron is a plant protection product that is used as a fungicide on potatoes as a seed treatment.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

The physico-chemical properties of pencycuron were assessed in the Draft Assessment Report and Proposed Decision of The Netherlands prepared in the context of the possible inclusion of pencycuron in Annex I of Council Directive 91/414/EEC (resubmission Draft Assessment Report, November 2009, RMS The Netherlands) concerning the placing of plant protection products on the market.

Pencycuron has no explosive properties as shown in the EEC A.14 study, is a solid and has no auto-ignition properties, is not flammable in contact with water and the molecular structure does not indicate oxidizing properties (table 8). Therefore, no classification of pencycuron for physico-chemical properties is required.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

See summary below.

4.1.2 Human information

No data available.

4.1.3 Summary and discussion on toxicokinetics

Oral absorption was determined in three different studies, with three different radiolabels, 6 different doses and using three different vehicles. All doses were administered by gavage and the % absorption was based on radiolabel from urine and, in the study by Ecker *et al.* (1989) only, in tissues (although the amount was negligible in comparison with the amount recovered from urine) and bile. An overview is presented in the table below). It appears that at comparable doses, oral absorption of the ^{14}C-methylene-(labelled) pencycuron is lowest, although an effect of the dosing suspension cannot be excluded. An indication for this is the fact that in the study in which Tragacanth was used as dosing suspension (study 1), the amount of unchanged pencycuron found in the faeces was much higher (ca. 50% of the administered dose of 2 mg/kg bw/d and ca. 75% after 100 mg/kg bw/d) than in the study with employed N,N-dimethylformamide in polyethylene glycol 400 as dosing suspension (17% after a dose of 40 mg/kg bw/d), suggesting that Tragacanth reduced the bioavailability of pencycuron. Furthermore, relative oral absorption decreases with increasing dose for each of the different radiolabels administered.

Table 9 Oral absorption of radiolabel from pencycuron in rats

Dose (mg/kg bw)	frequency	Label	% oral absorption	Strain	Vehicle	Reference
100	single	^{14}C-methylene	4.1	Wistar	0.5% Tragacanth TM	Ecker *et al.*, 1989

Dose (mg/kg bw)	frequency	Label	% oral absorption	Strain	Vehicle	Reference
2	repeated	^{14}C-methylene	11	Wistar	0.5% Tragacanth TM	Ecker *et al.*, 1989
2	single	^{14}C-methylene	7.1	Wistar	0.5% Tragacanth TM	Ecker *et al.*, 1989
200	single	^{14}C-carbonyl	ca. 33	Fischer	N,N-dimethyl-formamide in polyethylene glycol 400 (1:9 v/v)	Oyama *et al.*, 1982
200	single	^{14}C-phenyl	19-13 (m-f)	Fischer	N,N-dimethyl-formamide in polyethylene glycol 400 (1:9 v/v)	Oyama *et al.*, 1982
40	single	^{14}C-carbonyl	ca. 33	Fischer	N,N-dimethyl-formamide in polyethylene glycol 400 (1:9 v/v)	Oyama *et al.*, 1982
40	single	^{14}C-phenyl	31-53 (m-f)	Fischer	N,N-dimethyl-formamide in polyethylene glycol 400 (1:9 v/v)	Oyama *et al.*, 1982
5	single	^{14}C-phenyl	21	Sprague-Dawley	rat feed slurry	Chopade & Bailey, 1986
500	single	^{14}C-phenyl	5	Sprague-Dawley	rat feed slurry	Chopade & Bailey, 1986
2	single	^{14}C-methylene	42 excreted via bile + 3.8 via urine	Wistar	0.5% Tragacanth TM	Ecker *et al.*, 1989

The study by Ecker *et al.* (1989) is better reported than the study by Oyama *et al.* (1982) and more complete than the study by Chopade & Bailey (1986)[1]. Also in the study of Oyama *et al.* (1982) no dose which was non-toxic in repeated dose studies, was tested.

Based on experiments with bile duct cannulated male rats executed by Ecker *et al.* (1989), and including radiolabel recovered from bile into the absorbed pool, absorption from the gut after single oral low dose was 46%, 48 h after administration. As there are distinct indications for a sizeable biliary first-pass effect (see section below on excretion), a significant part of the biliary radiolabel may not represent systemically available parent compound and/or metabolites. The other authors did not report bile cannulation experiments.

In conclusion, in rats, oral absorption of radiolabelled pencycuron is at least 7.0% after single low dose, at least 11 % after repeated low dose and at least 4.0% after single high dose when the excretion via bile is not taken into account.

[1] The study by Oyama *et al.* (1982) is part of a series of toxicokinetic studies. Other parts of this series were reported by Kobori *et al.* (1982) and Hoshino *et al.* (1982).

A special study with rats indicated that the presence of clay in the diet does not alter the bioavailability of radiolabel after single oral administration of [UL-^{14}C-phenyl]pencycuron (5 and 500 mg/kg bw/d).

An oral absorption of 46% was determined for the derivation of the AOEL based on the amount of substance and metabolites that reached the target organ (liver).

Excretion

In rats, after oral administration of pencycuron radiolabelled at the methylene-, carbonyl- and phenyl-group[2] excretion of radioactive carbon dioxide was negligible. Three days after administration of [N-^{14}C-methylene]pencycuron, urinary excretion was 7.0-14% after single low dose (2 mg/kg bw/d), 11-19% after repeated low dose and 4.0-4.4% after single high dose (100 mg/kg bw/d) in male and female rats. Total urinary and faecal excretion within three days after administration was 84-92% of the administered dose (m-f) after single low dose, 76-91% after repeated low dose and 86-85% after single high dose. For all dose regimens excretion was nearly completed within 24 h, as more than 90% of the amount excreted within three days had been eliminated in the first 24 hours. After a single low dose of [N-^{14}C-methylene]pencycuron, 42% of the administered radiolabel was excreted in bile, within 48 h after administration. Some enterohepatic circulation took place, but ca. three quarters of the biliary radiolabel (eventually) is excreted in the faeces. Total excretion after oral administration of [UL-^{14}C-phenyl]pencycuron or [^{14}C-carbonyl]pencycuron showed a comparable time course, although the balance between urinary and faecal excretion was different. For the reasons mentioned in the section “Absorption”, the RMS prefers the study with [N-^{14}C-methylene]pencycuron executed by Ecker *et al.* (1989).

Distribution

After oral administration of 2 (single and repeated) or 100 (single) mg/kg bw/d [N-^{14}C-methylene]pencycuron, the amount of radiolabel retained in the body, three days after administration, was relatively small for all dose groups: 0.22% of the administered dose or less. The concentration of radioactivity was highest in the liver (0.030-0.024 mg eq./kg (m-f) after single low dose, 0.041-0.041 mg eq./kg after repeated low dose and 0.14-0.092 after single high dose). In the other tissues concentrations were lower by a factor 3 or more. In all dose groups the concentration of radioactivity was a factor 6-15 higher in erythrocytes than in plasma. Once in the plasma, radiolabel is rapidly distributed in the body. The combination of the rather large terminal elimination half-lives (27-43 h) observed in plasma and the rather large volume of distribution may indicate distribution of radiolabel into deep compartments (e.g. binding to proteins).

After oral administration of [UL-^{14}C-phenyl]pencycuron or [^{14}C-carbonyl]pencycuron to rats also the liver was the organ with highest concentration of radiolabel. At comparable doses, concentration of radiolabel in the tissues and organs was higher than with the ^{14}C-methylene-label, which is consistent with the lower absorption recorded after oral administration of the latter radiolabel. For the reasons mentioned in the section “Absorption”, the RMS prefers the study with [N-^{14}C-methylene]pencycuron executed by Ecker *et al.* (1989). A distribution experiment with male mice in which UL-^{14}C-

[2] From the reports by Oyama *et al.,* Kobori *et al.* and Hoshino *et al.* (all 1982) it is not clear in which phenyl-group (the chlorobenzyl- or the phenylurea-group) the radiolabel was introduced. The summary of the notifier indicates this was the phenylurea-group.

phenyl]pencycuron was orally administered, showed high concentrations of radiolabel in the gall bladder (ca. 85x than in the liver, at a maximum, 8 hours after oral administration), indicating that also in this animal the bile is an important route of elimination.

Metabolism

In rats, once absorbed, pencycuron is nearly completely metabolised as after i.p. administration of [UL-^{14}C-phenyl]pencycuron only 0.1% of the administered radiolabel (40 mg/kg bw/d) was recovered from urine as unchanged parent compound, while in faeces it accounted for 1% of the administered radiolabel. Apart from the high presence of unaltered pencycuron in faeces after oral administration (17%), metabolite patters in faeces and urine are similar after oral an i.p. administration. After oral administration unchanged pencycuron was not detected in urine or accounted for less than 1% of the administered dose.

Repeated oral administration of pencycuron seems to induce liver metabolism, as after repeated low dose (2 mg/kg bw/d) of [N-^{14}C-methylene]pencycuron less unchanged pencycuron was recovered from faeces. In a special studies with rats on the induction of specific liver enzymes, repeated oral administration of pencycuron caused an increases in p-nitroanisole-O-demethylase activity in male but not in female rats after doses of 100 and 1000 mg/kg bw/d. Doses of 10 mg/kg bw/d did not have effects on the cited enzyme activities in either sex. In another similar study with only male rats, repeated oral administration of 1000 mg/kg bw/d pencycuron caused a slight increase in aminopyrene N-demethylase, EPN detoxicase and aniline hydroxylase activities in the liver, but a dose of 100 mg/kg bw/d did not. In short, indications for enzyme induction by oral administration of pencycuron are present, but not conclusive for low doses.

After oral administration of [N-^{14}C-methylene]pencycuron to rats, the most prominent metabolites identified in urine were 4-chlorohippuric acid (=M12), accounting for 0.9-4.0% of the recovered radiolabel and the glucuronide of M932(=M08-glc)[3] (0.5-3.7%). Other metabolites identified in urine were ECW6462 (=M05-glcm) and M932 (=M08), each accounting for less than 1% of the recovered radiolabel, and the glucuronide of M929 (=M05-glc) (not quantified). After oral administration of [UL-^{14}C-phenyl]pencycuron (40 mg/kg bw/d), the most prominent urinary metabolite was M932 (=M08) (mostly as sulphate conjugate), accounting for 13% of the administered dose. Other conclusively identified urinary metabolites were M929 (=M05) (1.1%), M942 (=M07-cis) (trans, 0.5%), 1-(p-chlorobenzyl)-3-phenylurea (=M02) (0.4%) and 1-(p-chlorobenzyl)-3-(4-hydroxy, 3-methylthiophenyl)urea (=M30) (0.1%). After oral administration of [^{14}C-carbonyl]pencycuron a similar metabolite pattern was found. No apparent explanation can be offered for main quantitative and qualitative difference between the metabolism of pencycuron after administration of [N-^{14}C-methylene]pencycuron on the one hand and of [UL-^{14}C-phenyl]pencycuron or [^{14}C-carbonyl]pencycuron on the other: the presence of M932 (=M08) as glucuronide or sulphate conjugate. Due to position of the label the metabolite 4-chlorohippuric acid (=M12) could not be detected after oral administration of [UL-^{14}C-phenyl]pencycuron.

[3] For explanation of the codes used, see list of identified compounds below.

After oral administration of [N-^{14}C-methylene]pencycuron to rats, the most prominent metabolites identified in faeces was N-(4-chlorobenzyl)-N-cyclopentylamine (=M16; = PB-amine; 8-10% of the recovered radiolabel in all dose groups).

There is sufficient evidence that this metabolite is formed in the liver and excreted via bile finally ending up in the feces and that it is not formed in the gut from active substance. The evidence is derived from the following data:

In the single oral dose of 2 mg/kg bw study (Ecker et al. 1989) 77 and 78 % (male and female) of the applied radioactivity is observed in the feces. In these feces 52 and 45% (male and female) of the applied dose was identified as active substance. As known from the Hoshino et al. (1982) report on the i.p. administered dose practically almost no active substance is excreted via the feces (1.1 %) and urine (0.1 %). All the systemic circulating dose was converted to metabolites and excreted via bile and then feces or directly via urine. Consequently, it is allowed to conclude that the active substance observed in the feces of intact animals remained during the entire passage through the animals in the gastrointestinal tract and was **not** absorbed. In the bile cannulation experiment (Ecker et al. 1989) only 50% of the applied radioactivity is observed in the feces. These 50% radioactivity (not analysed for metabolite pattern) correspond very well with the 50 % active substance in the feces of the intact animals. There is no room in the radioactivity balance of feces from bile cannulated animals for metabolites. The entire radioactivity of 50 % of the dose are most probably represented by intact active substance. Correspondingly, it is allowed to conclude that all the metabolites observed in the feces of intact animals have been formed in the liver and were excreted via bile into the intestine ending up in the feces. Therefore it is highly likely that also the most prominent metabolite in feces M16 (= THS1787. = PB-amine = N-(4-chlorobenzy)-N-cyclopentylamine) representing 8-10% of the applied dose was formed in the liver or even during systemic circulation.

The other identified metabolites were M941 (=M07-trans), M942 (=M07-cis), M932 (=M08) and M929 (=M05) each accounting for 1-4% of the recovered radiolabel after low dose (single or repeated) and for 0.4-1% after single high dose. After oral administration of [UL-^{14}C-phenyl]pencycuron (40 mg/kg bw/d), the most prominent faecal metabolite was M932 (=M08) (mostly as sulphate conjugate), accounting for 7.0% of the administered dose. Other conclusively identified faecal metabolites were M929 (=M05), M942 (=M07) (cis and trans), 1-(p-chlorobenzyl)-3-phenylurea (=M02) and 1-(p-chlorobenzyl)-3-(4-hydroxy, 3-methylthiophenyl)urea (=M30), all accounting for 1 to 2.5% of the administered dose. Due to position of the label the metabolite PB-amine (=M16) could not be detected after oral administration of [UL-^{14}C-phenyl]pencycuron or [^{14}C-carbonyl]pencycuron.

List of identified compounds

Below only the unambiguously identified parent compound and metabolites are listed. All compounds were identified *in vivo* in the rat.

Codes	Compound	Matrix [Urine/Faeces]
NTN 19701	pencycuron	F
ECW6462 (=M05glcm)	additional methoxy group attached to M05-glc	U
VII, M941 (=M07-trans)	1-(p-chlorobenzyl)-1-(3-hydroxycyclopentyl-3-(p-hydroxyphenyl)urea (trans)	F,U
VII, M942 (=M07-cis)	1-(p-chlorobenzyl)-1-(3-hydroxycyclopentyl-3-(p-hydroxyphenyl)urea (cis)	F
V, M929 (=M05)	1-(p-chlorobenzyl)-1-cyclopentyl-3-(p-hydroxyphenyl)urea	F,U
M929-glucuronide (=M05-glc)	1-(p-chlorobenzyl)-1-cyclopentyl-3-(p-hydroxyphenyl)urea glucuronic acid conjugate	U
XII (=M30)	1-(p-chlorobenzyl)-3-(4-hydroxy, 3-methylthiophenyl)urea	F,U
VIII, M932 (=M08)	1-(p-chlorobenzyl)-3-(p-hydroxyphenyl)urea	F,U
M932-glucuronide (=M08-glc)	1-(p-chlorobenzyl)-3-(p-hydroxyphenyl)urea glucuronic acid conjugate	U
II (=M02)	1-(p-chlorobenzyl)-3-phenylurea	F,U
(=M12)	4-chlorohippuric acid	U
THS1787, PB-amine (=M16)	N-(4-chlorobenzyl)-N-cyclopentylamine	F

Dermal absorption

A dermal absorption study with technical pencycuron is not available.

An in-vitro dermal absorption study with two formulation of pencycuron was conducted according to the draft OECD guideline 428 (2000). Epidermal membranes from female human and male rat were used and the formulation with 23.2% [methylene ^{14}C]- labelled pencycuron was applied undiluted (2.7 mg/cm^2) and diluted with water (0.13, and 0.71 mg/cm^2). The formulation with 12.5% pencycuron was applied as supplied (powder) at 0.19 mg/cm^2. A positive control was included. Samples of the receptor fluid were taken at 1, 2, 4, 6, 8, 10, 20, 22, and 24 hours after application. Radioactivity was analysed by LSC. In all experiments with both rat and human membranes the absorption rate of pencycuron did not decline during the 24h exposure period. No data elucidation the absorption rates after termination of the exposure are available. Therefore, it can not be excluded that radiolabel remaining in the membranes was systemically available.

Table 10: Distribution and recovery of radioactivity 24h after topical application of pencycuron in two different formulations to rat epidermis (% of applied dose).

Dose pencycuron [Monceren formulation]	0.13 mg/cm^2 [250 SC] mean ± SD	0.71mg/cm^2 [250 SC] mean ± SD	2.7 mg/cm^2 [250 SC] mean ± SD	0.19 mg/cm^2 [12.5 DS] mean ± SD
skin wash	80 ± 24	88 ± 6.2	90 ± 1.5	85 ± 14
epidermis, ring	2.3 ± 2.5	0.96 ± 1.3	0.26 ± 0.11	1.3 ± 0.67
absorbed (receptor fluid)	0.86 ± 0.15	0.10 ± 0.03	0.07 ± 0.04	0.27 ± 0.06
potentially absorbed dose[1)]	**3.2**	**1.1**	**0.33**	**1.6**
Total recovered	**84 ± 22**	**89 ± 6.6**	**90 ± 1.5**	**86 ± 14**

[1] receptor fluid, epidermis and fixing rings (calculated by the reviewer)

Table 11: Distribution and recovery of radioactivity 24h after topical application of pencycuron in two different formulations to human epidermis (% of applied dose).

Dose pencycuron [Monceren formulation]	0.13 mg/cm² [250 SC] mean ± SD	0.71mg/cm² [250 SC] mean ± SD	2.7 mg/cm² [250 SC] mean ± SD	0.19 mg/cm² [12.5 DS] mean ± SD
skin wash	92 ± 4.3	78 ± 5.2	94 ± 8.6	82 ± 10
epidermis, ring	2.9 ± 1.0	3.1 ± 1.9	1.3 ± 0.95	4.2 ± 2.8
absorbed (receptor fluid)	0.35 ± 0.10	0.07 ± 0.02	0.03 ± 0.00	0.30 ± 0.12
potentially absorbed dose[1)]	**3.3**	**3.2**	**1.3**	**4.5**
Total recovered	**95 ± 4.5**	**81 ± 5.3**	**95 ± 8.7**	**86 ± 12**

[1)] receptor fluid, epidermis and fixing rings (calculated by the reviewer)

The recovery rates varied between 81 – 95% and were in half of the cases clearly below the limit of 90%. Regarding this and the results obtained, this study can not be used to evaluate in detail the potential dermal absorption of pencycuron. However, the study is considered acceptable for the determination of the overall dermal absorption of pencycuron, as the differences in recovery are most likely due to differences in the efficacy in the extraction of the cotton swabs (skin wash fraction).

The potential absorption of pencycuron by human epidermal membranes is ca. 2-3 times higher than by rat epidermal membranes.
The potential absorption for pencycuron as 23.2% formulation by rat membranes accounts for ca. 2% at concentrations <1 mg/cm2 and for ca. 0.5% at concentrations of ca. 3 mg/cm2. For human membranes the potentially absorbed dose after administration of pencycuron as 23.2% formulation accounts for ca. 3% at concentrations <1 mg/cm2 and for ca. 1.5% at concentrations of ca. 3 mg/cm2. Pencycuron as 12.5% formulation was tested at only one concentration. Therefore it can only be concluded that the potentially absorbed dose of pencycuron as dustable powder at a concentration of ca. 0.2 mg/cm2 accounts for 1.6% and 4.5% for rat and human membranes, respectively.

A second in-vitro dermal absorption study with the formulation of 12.5% [^{14}C- labelled] pencycuron, was conducted according to the draft OECD 428 guideline with rat and human skin, Feurtet, 2005. The formulation was applied undiluted at 5 mg/cm^2 for both rat and human. The overall mean recovery of the dose to rat and hyman skin was 91.05 and 94.17%, respectively. In all experiments with both rat and hyman membranes, the absorption rate of pencycuron did not decline during 8 hours of exposure and 24 hours study period.

The potential absorption of pencycuron by rat epidermal membranes is approximately 10 times higher than by human epidermal membranes. The potential absorption for the undiluted 12.5% formulation of pencycuron accounts of 2.2% and 0.2% for rat and human membranes, respectively.

OVERALL CONCLUSIONS

Absorption from the gut is 46% in 48h after single low dose (2 mg/kg bw/d), based on radiolabel recovered from urine, tissues and bile. Oral absorption of pencycuron in rats is at least 7.0% after single low dose (2 mg/kg bw/d), at least 11 % after repeated low dose and at least 4.0% after single high dose (100 mg/kg bw/d), based on radiolabel recovered from urine, tissues and organs.

There are indications of a considerable first pass effect, as biliary excretion is high (40%). However, after i.p. or oral administration metabolite patterns are similar, apart from the higher percentage of

unaltered pencycuron in the faeces after oral dosing. This indicates that, in spite of the first pass effect, internal exposure to metabolites via the different portals of entrance (oral, inhalatory, dermal) may not be qualitatively different. However, in view of the large metabolic first pass effect, internal exposure to parent compound may be significantly different between the various routes. Absorption from the gut is 46% in 48h, based on radiolabel recovered from urine, tissues and bile.

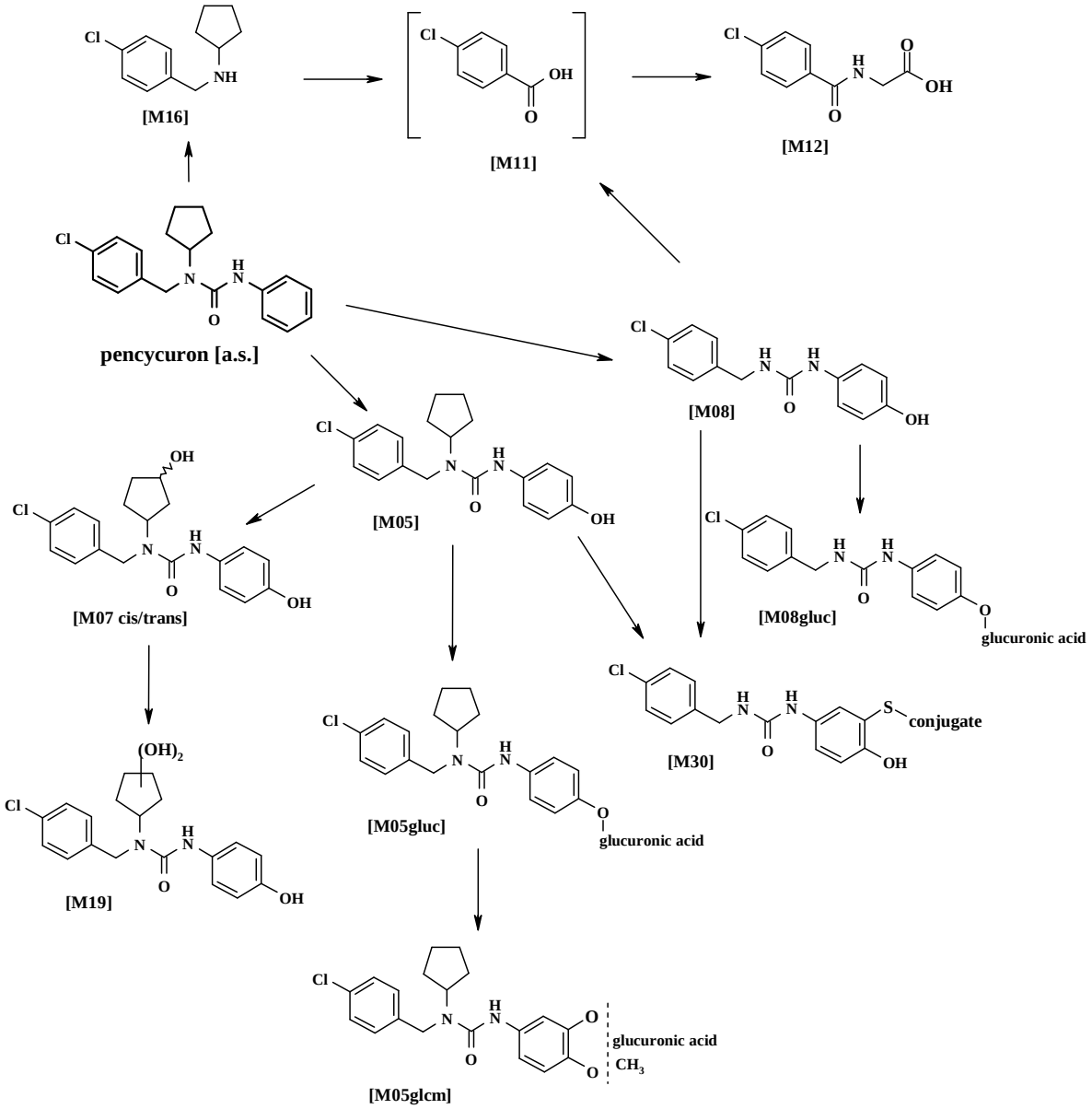
When deriving internal values for NOAELs from repeated dose oral toxicity studies, an oral absorption value of 11% (excluding bile excretion) should be used if the critical effect was observed in a post-hepatic organ or tissue, and a value of 46% (including bile excretion) if the critical effect was observed in the liver.

In rats, after oral administration of pencycuron excretion of radioactive carbon dioxide was negligible. Three days after administration of [N-^{14}C-methylene]pencycuron, urinary excretion was 7.0% after single low dose (2 mg/kg bw/d), 11% after repeated low dose and 4.0% after single high dose (100 mg/kg bw/d). Total urinary and faecal excretion within three days after administration was 84-92% of the administered dose (m-f) after single low dose, 76-91% after repeated low dose and 86-85% after single high dose. For all dose regimens excretion was nearly completed within 24 h.

After oral administration in the rat, little radiolabel is retained in tissues and organs, the highest concentrations are found in the liver. Once absorbed, pencycuron is nearly completely metabolised. Main urinary metabolites are 4-chloro-hippuric acid and the glucuronide and/or sulphate conjugate of M08 (M932).
After oral administration of [N-^{14}C-methylene]pencycuron to rats, the most prominent metabolites identified in faeces was N-(4-chlorobenzyl)-N-cyclopentylamine (= M16) (PB-amine; 8-10% of the recovered radiolabel in all dose groups).

There is sufficient evidence that this metabolite is formed in the liver and excreted via bile finally ending up in the feces and that it is not formed in the gut from active substance.

Remark: It is proposed to delete the arrow in the degradationscheme below from a.s. to 4-chlorohippuric acid (M12), since the degradation and the synthetic step seem to be less likely in one step.



M05 = NTN 19701 hydroxy-phenyl

M07 = NTN 19701 3-hydroxy-pentyl-4-hydroxy-phenyl

M08 = NTN 19701 hydroxy-descyclopentyl

M11 = 4-chloro-benzoic acid

M12 = 4-chloro-hippuric acid

M16 = NTN 19701 PB-amine

M19 = NTN 19701 di-hydroxy-pentyl-4-hydroxy-phenyl

M30 = NTN 19701 S-methyl-hydroxy-phenyl

RAC evaluation of physical hazards
Summary of the Dossier submitter's proposal No classification is proposed by the Dossier Submitter (DS) for physical hazards considering that pencycuron has no explosive properties as shown in an EEC A.14 study. Pencycuron is a solid and has no auto-ignition properties, it is not flammable in contact with water and the molecular structure does not indicate oxidizing properties. **Comments received during public consultation** No specific comments were received. **Assessment and comparison with the classification criteria** RAC supports the proposal of the DS not to classify pencycuron for physical hazards.

4.2 Acute toxicity

Table 12: Summary table of relevant acute toxicity studies

Method	Results (LD_{50} / LC_{50})	Remarks	Reference
Oral Toxicity			
OECD 401, gavage	> 5000 mg/kg bw	Rat	DAR (Sheets, 1989)
Aerosol Inhalation Toxicity			
OECD 403	> 5.13 mg/L	Rat	DAR (Pauluhn, 1990)
Dermal Toxicity			
Partly, OECD 402	> 2000 mg/kg bw	Rabbit	DAR (Sheets, 1988)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

One oral acute toxicity study in accordance with OECD 401 is available (Sheets, 1989). Pencycuron was administered in polyethylene glycol 400 in a limit test at 5000 mg/kg bw. Groups of 5 rats per sex were treated once orally, by gavage. No mortality was recorded and signs of toxicity were observed in 3 males and 1 female (urine stain) and in one male anal stain was found. All animals recovered within 3 days. One female lost body weight from day 7 to 14. No pathological findings in any animal. The following LD_{50} was established by this limit test > 5000 mg/kg bw, irrespective of sex.

Another two acute oral studies with rats are also available. However, both studies are not considered acceptable as the studies are not performed according OECD 401 guideline or were partly in accordance with the OECD 401.

In the first, limit, acute toxicity study rats were administered with pencycuron (99.0% purity) per gavage (Ono and Iyatomi, 1978).

The study was not performed in accordance with OECD 401, however, the guideline was not available at the time the study was conducted. The most important deviations from the guideline are:
• a dose level of 1000 mg/kg bw was used, while a dose level of 2000 mg/kg bw should be used in limit tests,
• the observation period was 7 days instead of 14 days,
• the study report was very concise.

The second unacceptable acute oral toxicity study with rats administered with pencycuron (purity 97.9%) is available (Kawaguchi et al. 1979).

The study was performed partly in accordance with OECD 401, however, the guideline was not available at the time the study was conducted. The most important deviation from the guideline is the observation period of 7 days instead of 14 days. Moreover, the study report was very conscise and body weight was not determined.

One acute oral study with dogs and cats was also performed. This study is also not considered acceptable as the study is not performed according to OECD 401.

In the last available acute oral toxicity study dogs and cats were administered with pencycuron (purity 99.4%) per gavage (Thyssen et al, 1980).

The study was not performed in accordance with OECD 401, however, the guideline was not available at the time the study was conducted. The study report was very concise. Presumably, pathological examinations were not performed as no pathology report was provided. The number of animals was very limited, which hampers the derivation of an LD50. As an additional effect parameter, the methaemoglobin concentration in the blood of cats was measured at 3, 5, and 24 hours and 2, 3, and 7 days after administration of the test substance.

4.2.1.2 Acute toxicity: inhalation

This study (Pauluhn, 1990) was performed in accordance with OECD 403. Groups of 5 males and 5 females were treated in a limit test with aerosol (particle size: MMAD 5.61 µm) by a head/nose exposure at 5.13 mg/L for 4 hours. This resulted in no mortalities, no treatment-related symptoms of toxicity and no findings related to pathology. Decreased body weight gain was observed in males and females on day 3. The following LC_{50} was established by this limit test > 5.13 mg/L, irrespective of sex.

Another study is available. Rats were exposed to pencycuron (purity 99.4%) for 1 hour or 4 and 5 x 6 hours at different concentrations (Thyssen et al, 1980). However, this study is not considered to be acceptable. The study was not performed in accordance with OECD 403, however, the guideline was not available at the time the study was conducted. The study report was very concise and lacked essential information (e.g. constancy of the air flow rate, particle size distribution, relative humidity). Except for body weight, no individual data were given. The highest test concentration used was the maximum producible concentration of the test substance.

4.2.1.3 Acute toxicity: dermal

This study (Sheets, 1988) was performed partly in accordance with OECD 402. The most important deviation from the guideline was that an occlusive dressing instead of a porous gauze dressing was

used. As the use of an occlusive dressing would increase the effects, this deviation is not considered to have influenced the conclusions of the study. Pencycuron, moistened with tap water, was applied to 5 male and female rabbits (New Zealand White) at a dose level of 2000 mg/kg bw in a limit test. One male animal died, which was related to a bacterial infection. Lacrimation was observed in one male and one female on day 1. No changes in body weight were found. Lesions indicative of a bacterial infection were found in the male that died. Bilateral lacrimation was found in one male. The following acute dermal LD_{50} was established: LD_{50} > 2000 mg/kg bw for both males and females.

Another two acute dermal studies with rats (and mouse) are also available. However, both studies are not considered acceptable, as the studies are not performed according OECD 402 guideline.

In one study (Ono and Lyatomi, 1978) rats were dermal exposed to pencycuron (purity 99.0%). The study was not performed in accordance with OECD 402, however, the guideline was not available at the time the study was conducted. The study report was very consice and was lacking essential information such as:

• application duration,
• body surface exposed to the test substance.
Other main deviations from the guideline were:
• non-occlusive exposure,
• observation period of 7 days.

The second unacceptable study (Kawaguchi et al, 1979) is performed with rats. Pencycuron (purity 97.9%) was dermal administered for 24 hours at one dose. The study was not performed in accordance with OECD 402, however, the guideline was not available at the time the study was conducted. The most important deviations from the guideline were:

• an observation period of 7 days instead of 14 days was used,
• the body surface area to which the test substance was applied was not specified,
• unclear whether or not the exposure was semi-occlusive,
• body weight was not determined,
• the study report was very concise.

4.2.1.4 Acute toxicity: other routes

No extensive summaries are provided as these routes do not lead to classification

Two acute intraperitoneal studies are available. In the first study (Ono and Lyatomi, 1978) both rats and mice were administered. The acute intraperitoneal LD_{50} was calculated to be 411 mg/kg bw for rats and 348 mg/kg bw for mice..

In a second, intraperitoneal study (Kawaguchi et al, 1979), groups of 10 male and female rats per dose were treated. The following acute intraperitoneal LD_{50} was calculated to be ca 1000 mg/kg bw for rats, irrespective of sex.

Two subcutaneous studies were also available.

In the first study (Ono and Lyatomi, 1978) both rat and mice were treated. The acute subcutaneous LD_{50} was found to be > 1000 mg/kg bw for rats and > 2000 mg/kg bw for mice, irrespective of sex.

In the second study only rats were treated (Kawaguchi et al, 1979). This study is acceptable as a supplementary study and the following LD_{50} was established: LD_{50} > 1000 mg/kg bw, irrespective of sex.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

Pencycuron has low acute oral, dermal, inhalation, intra-peritoneal and subcutaneous toxicity in rat and mice.. In the acute oral and inhalation toxicity studies no effects were found at > 5000 mg/kg bw and 5.13 mg/L, respectively. Dermal applied pencycuron (2000 mg/kg bw) caused lacrimation in some animals, which recovered during the study.

4.2.4 Comparison with criteria

Based on the limit test (Sheets, 1989) for oral acute toxicity on rats (LD50 > 5000 mg/kg bw) and according to the EC classification criteria (no classification for substances with an LD50 above 2000 mg/kg bw), pencycuron should not be classified for acute oral toxicity.

For acute inhalation, a 4-hour limit test using aerosol on rat is available showing no signs of toxicity and mortality at 5.13 mg/L (LC_{50} > 5.13 mg/L). Therefore, no classification for acute inhalation toxicity is required according to the EC classification criteria as the criteria state that substances should be classified if the LC50 for aerosols is at or below 5 mg/l in a 4-hour exposure study.

For the acute dermal toxicity, also a limit test is available showing no mortality was determined at 2000 mg/kg bw. LD50 was > 2000 mg/kg bw. According to the EC classification criteria (requiring classification if the LD50 is below 2000 mg/kg bw), pencycuron should not be classified for acute dermal toxicity.

4.2.5 Conclusions on classification and labelling

No classification for acute toxicity through the oral, dermal and inhalatory route is warranted based on the available information and the CLP criteria.

RAC evaluation of acute toxicity
Summary of the Dossier submitter's proposal
Acute toxicity: oral No classification was proposed based on the absence of mortality at the limit dose of 5000 mg/kg in a study on rats compliant with OECD TG 401. Signs of toxicity were observed in 3 males and 1 female (urine and/or anal stain). They recovered within 3 days. One female lost weight between day 7 to 14. In this study, the LD_{50} of pencycuron in rat by oral route exceeded 5000 mg/kg, irrespective of sex. The DS mentioned that additional oral acute toxicity studies were available on rats, dogs and cats but these studies were not considered acceptable due to deviations from the guidelines and were not further described. Acute toxicity: inhalation Rats (5/sex) were exposed (head-nose exposure) to 5.13 mg/L of pencycuron as aerosol

(MMAD 5.61 µm) for four hours in a study in accordance to OECD TG 403. No mortality, no clinical signs and no necropsy findings were observed. Decreased body weight gain was observed in males and females on day 3. In this study, the LC_{50} of pencycuron in rat by inhalation exceeded 5.13 mg/L, irrespective of sex.

The DS mentioned that an additional inhalation acute toxicity study was available on rats but this study was not considered acceptable due to limited reporting and was not further described.

Acute toxicity: dermal
Rabbits (5/sex) were exposed to a limit dose of 2000 mg/kg in a study partly compliant with OECD TG 402 (occlusive dressing to maximise the effect). One male died due to a bacterial infection. Lacrimation was observed in one male and one female on day 1. No effects on body weight were observed. In this study, the LD_{50} of pencycuron in rabbit by dermal route exceeded 2000 mg/kg, irrespective of sex.
The DS mentioned that additional dermal acute toxicity studies were available on rats and mice but these studies were not considered acceptable as they were not performed according to guidelines and were not further described.

No classification was proposed by the DS for acute toxicity.

Comments received during public consultation
Industry made some editorial comments on the description of the dermal acute toxicity study.

Assessment and comparison with the classification criteria
Acute toxicity: oral
Based on the data presented, the LD_{50} of pencycuron in rat is above the criteria of 2000 mg/kg, below which classification for acute toxicity by oral route applies according to CLP.

Acute toxicity: inhalation
Although it is noted that the mean particle diameter of the test substance (5.61 µm) is slightly outside the range of OECD TG 403 recommendation (1-4 µm), the study described provides no evidence that the LC_{50} of pencycuron in rats is below the criteria of 5 mg/L triggering classification for acute toxicity by inhalation for aerosols under CLP.

Acute toxicity: dermal
The LD_{50} of pencycuron in rabbit is above the criteria of 2000 mg/kg, below which classification for acute toxicity by dermal route applies according to CLP.

RAC supports no classification for acute toxicity as proposed by the DS.

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

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

In the acute oral toxicity study (Sheets, 1989), urine stain was found in three male and one female rats and one male showed anal stain. 5 rats per sex were orally exposed at 5000 mg/kg bw. All animals recovered within three days. Body weight loss was observed in one female from day 7 to 14.

In the acute inhalation study (Pauluhn, 1990), no treatment-related findings were observed. Rats (5/ sex/concentration) were exposed to pencycuron by head /nose inhalation for 4 hours at 5.13 mg/L. Body weight decrease was observed in males and females on day 3 only.

In the acute dermal toxicity study in rabbits (Sheets, 1988), lacrimation was observed in one male and one female on day 1 and in one male showed bilateral lacrimation at the limit dose of 2000 mg/kg bw.

4.3.2 Comparison with criteria

Classification with STOT SE category 1 or 2 is required when in an acute study significant toxicity is observed at dose levels at or below the guidance values of 2000 mg/kg bw (oral and dermal) or 5 mg/l/4 h (inhalation of aerosol). For category 3, evidence for respiratory tract irritation or narcotic effects should be present independent of the dose level. The effects observed in the available acute toxicity studies via the three different routes do not indicate a specific target organ toxicity after single exposure as the effects were limited at dose levels at or above the guidance value.

4.3.3 Conclusions on classification and labelling

No classification for STOT-SE through the oral, dermal and inhalatory route is required under CLP.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)
Summary of the Dossier submitter's proposal None of the findings reported further to single exposure following administration by oral, dermal and inhalation routes indicated a concern for specific target organ toxicity. No classification was proposed by the DS for STOT SE. **Comments received during public consultation** No specific comments were received. **Assessment and comparison with the classification criteria** No acute human data are reported and experimental data on animals do not indicate a target organ toxicity following acute exposure. RAC supports no classification for STOT SE, as proposed by the DS.

4.4 Irritation

4.4.1 Skin irritation

Table 13: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
OECD 404	Not irritating to skin	3 female rabbits	DAR 2009 (Maertins, 1989)

4.4.1.1 Non-human information

One study is available. Three female New Zealand White rabbits were treated with 0.5g technical test substance moistened with water during 4 hours semi-occlusive exposure and 7 days of observation. The study was performed in accordance to the OECD 404 guideline. Neither erythema nor oedema were observed at any observation time point until and including day 7. No other additional skin effects were observed in any animal.

Table 14 the mean scores of the skin irritation study in rabbits.

Scores observed after	1 hour	24 hours	48 hours	72 hours	7 days
Erythema	0,0,0,	0,0,0	0,0,0	0,0,0	0,0,0
Oedema	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0

Another skin irritation study on rabbits with pencycuron (purity 95%) with an exposure of 24 hours could not be properly evaluated because the study report was very concise and essential information was lacking. The study is unacceptable. The following information is lacking:

- the test concentration was not indicated,
- the surface area exposed to the test substance was not indicated,
- it was unclear whether or not exposure was semi-occlusive,
- observation period.

Therefore, the study could not be properly evaluated.

4.4.1.2 Human information

No data available.

4.4.1.3 Summary and discussion of skin irritation

One skin irritation study is available in which no skin irritation was found.

4.4.1.4 Comparison with criteria

Classification for skin irritation is required when in an OECD 404 study persistent effects or oedema or erythema at or above the time averaged grading of 2.3 is observed in two or more out of three rabbits, persistent inflammation (alopecia, hyperkeratosis, hyperplasia and scaling) in 2 out of 3 animals or very definite positive effects in a single animal. Pencycuron showed to be not a skin irritant as no such effects were observed.

4.4.1.5 Conclusions on classification and labelling

No classification for skin irritation is warranted based on the criteria of the CLP.

RAC evaluation of skin corrosion/irritation
Summary of the Dossier submitter's proposal In a study compliant with OECD TG 404, pencycuron (as powder moistened with water) was applied to the skin of three rabbits for 4 hours under semi-occlusive conditions. No irritation was observed at any time point (up to 7 days of observation) in any animal (scores of 0). Pencycuron was not irritating to the rabbit skin. The DS mentioned that an additional dermal irritation study was available with a 24-hour exposure, but this study was not considered acceptable due to limited reporting and was not further described. No classification was proposed by the DS for skin corrosion/irritation.

Comments received during public consultation
No specific comments were received.

Additional key elements
It was also noted that pencycuron did not induce any dermal effect in a 21-day repeated dermal study in which rabbits were exposed to pencycuron under semi-occlusive condition up to 1000 mg/kg (6h/d).

Assessment and comparison with the classification criteria
In the absence of any signs of irritation in a guideline study, pencycuron does not fulfil the criteria for skin irritation under CLP neither in terms of severity of scores nor in terms of irreversibility.
RAC supports no classification for skin corrosion/irritation, as proposed by the DS.

4.4.2 Eye irritation

Table 15: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
OECD 405	Not irritating to eyes	Three female albino rabbits	DAR 2009 (Maertins, 1989)

4.4.2.1 Non-human information

One eye irritation study is available. Three female New Zealand White rabbits were treated with 0.1 ml (50 mg) test substance by a single instillation of the eye. The study was performed according to the OECD 405 guideline. The treated eyes were washed 24 hours after instillation of the test substance. The scores observed after 1, 24, 48, and 72 hours and after 7 days were all zero for cornea/opacity, iritis, conjunctiva redness, conjunctiva chemosis and conjunctiva discharge.

Another eye irritation study is available. However, this study is not performed in accordance with OECD 405. This study is unacceptable. The study was not performed in accordance with OECD 405, because the study report was very concise and essential information (e.g. test concentration) was not provided. The eyes were exposed for 5 minutes and 24 hours.

4.4.2.2 Human information

No data available.

4.4.2.3 Summary and discussion of eye irritation.

One eye irritation study is available in which no eye irritation was observed.

4.4.2.4 Comparison with criteria

Classification as an eye irritant is required if 2 or more out of three rabbits in an OECD 405 show a time averaged grading of cornea opacity ≥1, iritis ≥1, conjunctival redness ≥ 2 or conjunctival oedema

≥2. Pencycuron showed to be not an eye irritant as no effects on the eye were observed in an OECD 405 study.

4.4.2.5 Conclusions on classification and labelling

No classification for eye irritation is warranted based on the criteria of the CLP.

4.4.3 Respiratory tract irritation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015. For assessment of this effect under CLP and the relevant acute inhalation studies see section 4.3.

4.5 Corrosivity

4.5.1 Non-human information

No specific studies are available. In the skin irritation study no irritation nor any skin destruction was observed.

4.5.2 Human information

No data available.

4.5.3 Summary and discussion of corrosivity

In the skin irritation study no corrosive effects of the skin were found.

4.5.4 Comparison with criteria

Classification as skin corrosive is required when skin corrosion is observed in a OECD 404 after exposure during 4 hours or less. Pencycuron showed to be not corrosive to skin as no corrosive effects were observed in a OECD 404 study.

4.5.5 Conclusions on classification and labelling

No classification for skin corrosion is warranted based on the criteria of the CLP

RAC evaluation of eye corrosion/irritation
Summary of the Dossier submitter's proposal In a study compliant with OECD TG 405, pencycuron was instilled into the conjunctival sac of three rabbits. No irritation was observed at any time point (up to 7 days of observation) in any animal (scores of 0). Pencycuron was not irritating to the rabbit eye. The DS mentioned that an additional eye irritation study was available but this study was not considered acceptable due to limited reporting and was not further described. No classification was proposed by the DS for eye irritation. **Comments received during public consultation** No specific comments were received.

Assessment and comparison with the classification criteria
In the absence of any signs of irritation in a guideline study, pencycuron does not fulfil the criteria for eye irritation under CLP neither in terms of severity of scores nor in terms of irreversibility.

RAC supports no classification for eye corrosion/irritation, as proposed by the DS.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 16: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
OECD 406	Not sensitizing to skin	Maximisation test	DAR 2009 (Heimann, 1984)

4.6.1.1 Non-human information

One study is available (Heimann, 1984). The study is partly performed according to the OECD 406 guideline. The following deviations from the guideline were: no results of a reliability check were provided, no results of the induction application were provided and the dose-selection is unclear.

Twenty male guinea pigs were tested and ten animals in the control group. Induction was intradermal and topical and an occlusive topical challenge of 24 hours. Vehicle used was Cremophor EL (a polyethoxylated castor oil, used to stabilise the emulsion) and physiological saline solution (0.2 mL/10 mL). The doses in the main study were based on the results of a range-finding study using 0, 1, 2.5, and 5% w/v (intradermal induction) and 3, 6, 12.5, and 25% w/v (topical induction). Although the effects induced by intradermal injection of the test concentrations were not indicated, the intradermal induction dose was determined to be 1% w/v. No abnormal reactions in the animals were observed after topical application of the test concentrations. Therefore the highest dose of 25% w/v was selected for both the induction and challenge exposure. 10% SLS in paraffin oil was used before the dermal induction. The test compound group consists of 20 animals and the control group of 10 animals. Sensitisation was determined by a dressing with test compound on the left flank of the animals in the test compound group and the control group. In addition, for comparison a similar control dressing was fastened to the right flank of all the animals (treated and control group), and this had only been soaked with the formulation vehicle.
Effects caused by intradermal injection and topical induction were not indicated. Following challenge with 25% w/v, dermal responses were observed on the left flank in 2/20 (10%) and on the right flank, the control flank, in 1/20 (5%) of the test animals treated with the test compound and vehicle, and in 0% of the negative control animals (0/10).

Although the highest topical induction concentration of 25% w/v did not result in a skin reaction, this concentration is considered to be sufficient, as it is near the maximum concentration for powders to obtain a well-mixed suspension (ca. 30%).

Another skin sensitization study, a Buehler test, is available (Sheets, L. P., 1989b). However, this study was not performed in accordance with OECD 406. The following deviations from the guideline

were observed: too few test and control animals were used, the choice for the vehicle is unclear, because it caused effects on the skin, the dose-selection is unclear, the challenge exposure (24 hours) was too long, and the control animals did not receive the induction application.

The doses in the main study were based on the results of a range-finding study using 0.5, 1.0, 5.0, 10, 25, 50, and 100% (i.e. moistened with water) w/v topical applications. The vehicle and all test concentrations except the 100% concentration caused slight irritation of the skin. It was unclear if good skin contact was achieved at the 100% concentration. The main test was performed with 50% w/v/ topical application. Animals in the test groups received three topical induction applications (6-hour duration each) on study days 0, 7 and 14, followed by a topical challenge application (24-hour duration) on day 27. Test group consisted of 15 animals, control of 5 animals. Reference substance used was DNCB (1-chloro-2,4-dinitrobenzene) and a control group, each 5 animals. No erythema or other skin adverse effects were observed in the pencycuron group. One animal in the reference group showed erythema and another animal showed eschar. The study is considered unacceptable, because of the deviations from the guideline.

4.6.1.2 Human information

No data available.

4.6.1.3 Summary and discussion of skin sensitisation

In a Maximisation skin sensitisation study (Heimann, 1984), 10% of the test animals showed a positive dermal reaction.

4.6.1.4 Comparison with criteria

Classification as a skin sensitiser is required when 30% or more of the animals in a Maximisation test show a positive response. This criterion is not fulfilled as only 10% of the animals showed a positive response.

4.6.1.5 Conclusions on classification and labelling

According to the CLP criteria, pencycuron should not be classified as a skin sensitizer.

RAC evaluation of skin sensitisation
Summary of the Dossier submitter's proposal In a Guinea Pig Maximization Test (GPMT) compliant with OECD TG 406, pencycuron was injected intradermally to 20 animals at a concentration of 1%. For both topical induction and challenge phases, the test article was dosed at a 25% concentration. Vehicle used was Cremophor EL (a polyethoxylated castor oil) and physiological saline solution. 10% sodium lauryl sulfate (SLS) was used before the dermal induction. Dermal responses were observed in 2/20 (10%) of the test animals. Challenge with vehicle only on the opposite flank of the test animals resulted in dermal responses in 1/20 (5%) of animals. No reaction was observed in control animals (0%). The DS also mentioned a Buehler test performed using a concentration of 50% pencycuron in ethanol: water (80:20) for both topical induction and challenge. No dermal response was observed in the test animals. The study was however considered unacceptable by the DS due to deviations from the guideline (small number of animals, unclear justification for the choice of vehicle and dose, 24h challenge exposure, no induction exposure in controls).

Based on the negative GPMT, no classification was proposed by the DS for skin sensitisation.

Comments received during public consultation
No specific comments were received.

Assessment and comparison with the classification criteria
Although it is noted that the use of higher topical concentrations should have been investigated, the result of the GPMT test does not fulfil the criteria of 30% of animals with a positive reaction that would indicate a skin sensitisation potential at the doses tested.
Although of limited quality, the Buehler test does not raise a concern regarding a skin sensitising potential.

On the basis of the available information, RAC therefore supports no classification for skin sensitisation.

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

No information available.

4.6.2.2 Human information

No information available

4.6.2.3 Summary and discussion of respiratory sensitisation

No information available

4.6.2.4 Conclusions on classification and labelling

No classification based on absence of data.

RAC evaluation of respiratory sensitisation

Summary of the Dossier submitter's proposal
No human or experimental data were available to assess respiratory sensitisation potential and no classification was proposed by the DS for respiratory sensitisation.

Comments received during public consultation
No specific comments were received.

Assessment and comparison with the classification criteria
In the absence of any relevant data, RAC considers that it is not possible to classify pencycuron for respiratory sensitization.

4.7 Repeated dose toxicity

Table 17: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
OECD 410	NOAEL 1000 mg/kg bw/d	21-day **dermal** toxicity study	DAR 2009 (Diesing, 1992)
OECD 408	NOAEL: 120 mg/kg bw/d	14 week (90 days) oral toxicity study in rats in diet	DAR (Inukai et al.1978a)
OECD 408	NOAEL: 65 mg/kg bw/d	13 weeks (90 days) oral dietary toxicity study in mice.	DAR (Inukai et al, 1978b)
OECD 409	NOAEL: 277 mg/kg bw/d	365 days oral dietary toxicity study in dogs.	DAR (Bathe et al, 1983)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

There are no acceptable 28 days repeated oral toxicity studies available

In a 14- week semi-chronic oral toxicity study (Inukai, 1978a) pencycuron (purity 98%) was administered by diet to rats at 0, 80, 400, 2000, and 10000 mg/kg food (equal to 0, 4.6, 24, 120, and 610 mg/kg bw/d for males and 0, 5.6, 28, 138, and 712 mg/kg bw/d for females) partly according to OECD 408 is available.15 animals/sex/does were used and an additional group of 5 animals/sex/does for blood and urine analysis. Body weight, food consumption, clinical chemistry, organ weights, urinalysis, and haematology were examined in 15 animals/sex/dose in week 14. Haematological examination, clinical chemistry examination and urinalysis were performed in week 4 and 8 with an addition group of 5 animals/sex/dose. For histopathology tissues from 10 animals/sex were examined.

Table 18 Results of a 14-week semi-chronic oral toxicity study in rats:

Dose (mg/kg food)	0		80		400		2000		10000		dr
	m	f	m	f	m	f	m	f	m	f	
Organ weights											
- liver					dc[r]		i[a], ic[r]	ic[r]	ic[a,r]	i[a], ic[r]	m,f
- adrenals									i[a]	ic[r]	
Pathology											
macroscopy	no treatment related effects										
microscopy											
liver											
- minimal change of polymorphism in the nucleus					+		+		++	++	m
- abnormal distribution of chromatin in the nucleus				+	+	+	+		++	++	m
- irregular nucleus in size									++	++	

Empty cells indicate no changes compared to controls
dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a,r absolute/relative organ weight
+ present in one/a few animals
++ present in several more animals

Dose (mg/kg food)	0		80		400		2000		10000	
	m	f	m	f	m	f	m	f	m	f
Body weight				-3%		-3%		-5%*		-6%*
liver										
absolute organ weight			-1%	-5%	-4%	0%	+5%	+0%	+10%*	+7%
relative organ weight			-3%	-3%	-5%*	+3%	+6%*	+6%*	+11%*	+14%*
minimal change of nuclear polymorphism					1/10		2/10		5/10	6/10
abnormal distribution of chromatin in the nucleus				1/10	1/10	2/10	2/10		5/10	6/10
irregular nucleus in size									5/10	6/10

Empty cells indicate no changes compared to controls
* statistically significant difference with control group

There were no mortalities. No treatment-related findings in food consumption, clinical signs, haematology, clinical chemistry and urinalysis. Oral treatment with pencycuron for 90 days resulted in effects on body weight and the liver. From a dose level of 2000 mg/kg food and upwards, the (relative) liver weights in males and females were significantly increased and female body weight significantly reduced in comparison to the control group. At a dose level of 400 mg/kg food up to the highest tested dose, microscopic findings in the livers of males and females included minimal change of polymorphism in the nucleus, abnormal distribution of chromatin in the nucleus and irregular nucleus size. These symptoms are regularly found in rats with a rather varying incidence. Only at the highest dose, there is a clear increase; the observed nuclear changes at the lower dose levels are considered to be a chance finding. The changes in (female) body weight and in liver weight observed at doses below 10000 are relatively small, and considered to be not (yet) adverse. Also the reduced body weight in the

highest female dose group is still relatively small. In conclusion, based on the observed liver effects, the NOAEL is set at 2000 mg/kg food, which is equal to 120 mg/kg bw/day.

The deviations from the guideline included: there was no ophthalmoscopy performed, no functional observational battery was observed, and there was an inaccurate reporting of the method and results. A detailed description of the pathological and histological findings was not written, only a few sentences of the findings had been reported also in the separate pathological report. Furthermore, as this study was performed at a time when the performance of ophthalmoscopy and of functional observational battery examinations were not required by the then existing guidelines, these deviations are not considered of relevance for the quality of this study.

In a 13-week semi-chronic oral toxicity study (Inukai, 1978b) pencycuron (purity 98%) was administered by diet to mice at 0, 80, 400, 2000, and 10000 mg/kg food (equal to 0, 9.7, 50, 264, and 1345 mg/kg bw/d for males and 0, 13, 65, 315, and 1552 mg/kg bw/d for females) partly according to OECD 408 is available. 20 animals/sex/dose were treated. For histopathology tissues from 10 animals/sex were examined.

Table 19 Results of a 13-week semi-chronic oral toxicity study in mice:

Dose (mg/kg food)	**0**		**80**		**400**		**2000**		**10000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Clinical chemistry											
- ALT (alanine transaminase)							ic		ic		
- LDH (lactic dehydrogenase)							ic		ic		
Organ weights											
- liver						ic^{r}		$ic^{a,r}$	$ic^{a,r}$	i^{a}, ic^{r}	
- spleen							ic^{r}		$ic^{a,r}$		
- submaxillary glands				$dc^{a,r}$		$d^{a,r}$		$dc^{a,r}$		$dc^{a,r}$	
- thymus				$dc^{a,r}$		$dc^{a,r}$		$dc^{a,r}$		$d^{a,r}$	
- kidney								dc^{r}		$dc^{a,r}$	
Pathology											
macroscopy	no treatment-related effects										
microscopy											
liver - minimal change of nuclear polymorphism									++	++	
- abnormal distribution of chromatin in the nucleus								+	++	++	

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a,r absolute/relative organ weight
\+ present in one/a few animals
++ present in several more animals

	males				females			
Dose (mg/kg food)	**80**	**400**	**2000**	**10000**	**80**	**400**	**2000**	**10000**
spleen								
absolute organ weight	+12%	+12%	+15%	+17%*	0%	+4%	-9%	+2%
relative organ weight	+13%	+11%	+16%*	+18%*	+2%	+6%	-11%	+4%
kidney								
absolute organ weight	-1%	+1%	+3%	-1%	-4%	-1%	-4%	-13%*
relative organ weight	-1%	+0%	+4%	-1%	-2%	+1%	-7%*	-10%*
liver								
absolute organ weight	+0%	+3%	+0%	+8%*	+4%	+6%	+10%*	+7%
relative organ weight	+0%	+2%	+2%	+9%*	+5%	+8%*	+7%*	+10%*
minimal change of polymorphism in the nucleus				4/10				5/10
abnormal distribution of chromatin in the nucleus				4/10			1/10	5/10
Thymus								
absolute organ weight	+6.5%	+2%	-4%	-9%	-21%*	-13%*	-19%*	-10%
relative organ weight	+5.5%	+1%	-3%	-11%	-19%*	-12%*	-21%*	-8%
Submxillary glands								
absolute organ weight	-4%	-3%	-3%	-1%	-14%*	-12%	-23%*	-23%*
relative organ weight	-4%	-3%	-1%	+0%	-13%*	-10%	-25%*	-22%*

* statistically significant difference with control group

No mortalities occurred. There were no treatment-related effects in clinical signs, body weight gain, food consumption, haematology and macroscopic pathology. Oral exposure of mouse to pencycuron for 90 days resulted mainly in effects on the liver. The changes of the weights of the submaxillary glands and thymus were actually observed in all female dose groups, but were however not always statistically significant. As there was no clear dose relation, effects on these organ weights were not considered related to treatment. The effects on spleen weight in males observed at all dose levels are probably due to the relatively low average of their control group. Also in the female group spleen weight is rather variable, in a not treatment-related fashion. Therefore, the effect on the spleen weight in males is not considered to be treatment related. The changes in kidney weight observed in females at doses of 2000 mg/kg food and higher are considered related to treatment. In view of the relatively low decrease at 2000 mg/kg food and in absence of histopathological findings, the kidney effect is not considered to be adverse in this dose group. From a dose level of 400 mg/kg and higher statistically significant increase of the (relative) liver weight was observed in the females. In view of the relatively low increase at 400 mg/kg food and in absence of histopathological findings, the liver effect is not considered to be adverse in this dose group. In the males only at the highest dose level of 10000 mg/kg food an increase in the (relative) liver weight was observed. In addition, microscopical changes in the liver included minimal change of polymorphism in the nucleus and abnormal distribution of chromatin in the nucleus of males and females at a dose level of 10000 mg/kg food. One female also showed these microscopical changes at dose level 2000 mg/kg food, but as they are regularly observed in mice with varying incidence this particular finding is considered incidental. In conclusion, the NOAEL is set at 400 mg/kg (equal to 65 mg/kg bw/d), based on liver effects first observed in females and at higher doses also in males

The deviations from the guideline included: there was no ophthalmoscopy performed, no functional observational battery was observed, and there was an inaccurate reporting of the method and results. A detailed description of the pathological and histological findings was not written, only a few sentences of the findings had been reported. Furthermore, as this study was performed at a time when the performance of ophthalmoscopy of functional observational battery examinations were not required by the then existing guidelines, these deviations are not considered of relevance for the quality of this study.

In a chronic oral toxicity study (Bathe et al, 1983) pencycuron (purity 99.2%) was given by dietary administration at concentrations of 0, 100, 1000, and 10000 mg/kg food (equal to 0, 2.7, 31, and 277 mg/kg bw/d for males and females) to groups of 6 dogs (beagle)/sex/dose for 1 year (minimum of 360 days). The study is in accordance with OECD 409. It was not specified on what basis the doses were selected. Haematological and clinical chemistry examination were performed before the treatment and 1, 2, 3, 4, 5, 6, 8, 10 and 12 months after initiation of treatment.

Table 20 Results of a chronic oral dietary toxicity study with dogs:

Dose (mg/kg food)	**0**		**100**		**1000**		**10000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Food consumption						d (ca. 9%)		d (ca. 9%)	
Liver enzymes									
- Cytochrome P-450					ic	ic	ic	ic	m,f
Organ weights									
- liver					ic[a], i[r]		ic[a], i[r]	i[a,r]	
Pathology									
macroscopy	No treatment-related effects								
microscopy	No treatment-related effects								

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a,r absolute/relative organ weight

Dose (mg/kg food)	**0**		**100**		**1000**		**10000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Liver enzymes								
- Cytochrome P-450 (nmol/g)	12.1	12.1	12.4	12.5	15.9*	16.7*	30.4*	28.2*
Organ weights								
- liver (relative) (%)	2.8	3.0	2.5	2.7	2.9	3.0	3.1	3.2
- liver (absolute) (g)	216	214	214	216	247*	216	253*	237
Body weight (kg)								
- initial	3.6	5.1	5.4	5.5	4.5	5.4	5.1	4.7
- terminal	8.2	7.9	8.8	8.5	8.9	7.7	8.6	7.8

* statistically significant difference with control group

There were no mortalities, no treatment-related effects were found for clinical signs, body weight gain, ophthalmoscopy, urinalysis, and haematology. The observed decrease in food consumption in the female high dose group is not considered adverse, as it is not statistically significant and did not result in a reduced body weight gain. The difference in absolute liver weight between the male mid and high dose group on the one hand and the male control group on the other, are most likely provoked by differences in body weight, already present at the start of the experiment. Differences in relative liver weight are small and not statistically significant. Also considering that no histopathological changes have been found in the liver, it is concluded that there are no clear adverse effects on this organ

weight. The clear, dose related increase in cytochrome P-450 content of both male and female livers is an adaptive reaction, and is not considered adverse since it is not associated with other, probably adverse, liver effects. As no adverse effects of treatment were observed, the NOAEL of this study is set at 10000 mg/kg food (277 mg/kg bw/d).

Supplementary studies

Two 28 days repeated oral toxicity studies are available, which are both considered to be acceptable as supplementary. Based on shortcomings in the design and / or reporting, no NOAEL could be determined for both studies.

In the first 28 day oral repeated toxicity study (Inukai et al, 1978a), 10 rats/sex/dose were administered via diet at 0, 100, 1000 and 10000 mg/kg food (equal to 0, 9.7, 100, and 1034 mg/kg bw/d for males and 0, 10, 102, and 1056 mg/kg bw/d for females).

Two additional groups were incorporated: one of 10 animals/sex at all dose levels and one of 10 animals/sex at dose levels of 0, 1000, and 10000 mg/kg food. These groups were included as satellite groups and observed for a 4- and 9-week treatment-free period after the main study, respectively, to assess the possible recovery or delayed occurrence of any toxic effects. However, all toxicity data of each of the satellite groups were reported separately and apart from the main study. Hence although during the first 4 weeks of the study 20-30 animals/sex/dose were treated, the results were reported and statistically analysed in groups of 10. Therefore, the data of the 10 animals/sex/dose of the main study (i.e. not the satellite groups) are summarised and evaluated below.

No mortalities, no treatment related clinical effects or haematology or findings for clinical chemistry were found. No effects in food consumption.

Table 21 Results of a 28-days oral dietary study with rats.[1]

.Dose (mg/kg food)	0		100		1000		10000		dr
	m	f	m	f	m	f	m	f	
Body weight			d	dc		d	dc	dc	
Body weight gain - week 1-4			d	dc		d	dc	dc	
Organ weights - brain							dc^{a}		
- liver					$ic^{a,r}$		$ic^{a,r}$	ic^{r}	
Pathology *Kidneys* - moderate to marked epithelial proliferation of pelvis					+	+		+	

1 results of the two satellite groups are not included
dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a,r absolute/relative organ weight
+ present in one/a few animals
++ present in most/all animals

The study was partly in accordance with OECD 407, however no OECD guideline was available at the time the study was conducted. There were major deviations from the guideline: information about the identification of the substance (batch, purity, physical nature) was lacking, no information was provided on the diet formulation, achieved concentrations, stability and homogeneity of the diet, it was

not reported how often clinical observations were made and no summary and individual data of the clinical observations and histopathological examinations (except for liver and kidney) were included.

Moreover, only at the highest test concentration clay (4%) was used as a vehicle, which may have altered the effects of the test substance. No reasoning for using clay is provided. The description of the statistical methods used was very concise, and it was unclear if the results obtained at the highest concentration were compared with those of the control or the vehicle-control group (5% clay).

Oral exposure of rats to pencycuron for 28 days resulted in reduced body weight and body weight gain in females exposed to concentrations of 100 mg/kg food and higher and in males exposed to 100 and 10000 mg/kg food. These effects were less pronounced in animals of the two satellite groups and mostly disappeared during the recovery period. Because the changes in body weight and body weight gain were less than 10% in all dose groups, except for the body weight gain in females of the highest dose group, they were not considered to be adverse. Absolute brain weight was decreased in males of the highest dose group In males, absolute and relative liver weight were increased at concentrations of 1000 and 10000 mg/kg food, while relative liver weight of females was increased at the highest dose. Changes in the kidneys increased in severity at the highest test substance concentrations (only in the main groups not in the recovery groups). Based on the short-comings in the design and reporting, no NOAEL could be determined and the study is considered to be supplementary.

In the second 28 days oral repeated toxicity study (Inukai et al, 1978b) 10 mice/sex/dose were tested at 0, 100, 1000, and 10000 mg/kg food (equal to 0, 14.5, 154, and 1559 mg/kg bw/d for males and 0, 15.6, 165, and 1758 mg/kg bw/d for females).

No mortalities, no treatment related effects in clinical signs, body weight, food consumption, haematology, and clinical chemistry.

Table 22 Results of a 28-days oral dietary study with mice[1]

Dose (mg/kg food)	**0**		**100**		**1000**		**10000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Organ weights									
- brain					ic^{r}		ic^{r}		m
- liver				i^{r}	ic^{r}		ic^{r}		m
- kidneys			dc^{ar}	dc^{r}	d^{a}		dc^{ar}	dc^{ar}	
Pathology									
Liver									
- foci of cell infiltration	0/10	1/10	0/10	3/10	0/10	3/10	1/10	2/10	
- irregular nucleus size	0/10	1/10	1/10	1/10	3/10	0/10	3/10	2/10	
Kidneys									
- epithelial proliferation of pelvis	2/10	1/10	1/10	3/10	2/10	2/10	4/10	3/10	

1 results of the two satellite groups are not included
dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a,r absolute/relative organ weight
+ present in one/a few animals

The study was performed partly in accordance with OECD 407, however, no OECD guideline was available at the time the study was conducted. The study design and deviations from the guideline were identical to that of the first study performed with rats (see study above).

Two satellite groups, one of 10 animals/sex at all doses and one of 10 animals/sex at dose levels of 0, 1000, and 10000 mg/kg food, were observed for a 4- and 10-week treatment-free period after the main

study, respectively, to assess the possible recovery or delayed occurrence of any toxic effects, the clinical chemical examination consisted only of biliburin, BUN (blood urea nitrogen), GOT (glutamic-oxaloacetate transaminase), and LDH (lactic dehydrogenase).

The study is of limited acceptability, because a detailed histopathological report is lacking and the results obtained for the three control groups showed a lot of variation. Therefore, no NOAEL can be determined and the study is considered to be supplementary.

Oral exposure of mice to pencycuron at concentrations of 0, 100, 1000, and 10000 mg/kg food for 28 days resulted in increased relative brain and liver weights in males exposed to 1000 and 10000 mg/kg food, but these changes were no longer present after a 4-week recovery period. Relative liver weight of females was decreased at a dose level of 100 mg/kg food, but this was not statistically significant. Absolute and relative kidney weights were decreased in males and females exposed to 10000 mg/kg food and in males exposed to 100 mg/kg food. At 100 and 1000 mg/kg food, relative kidney was decreased in females and a statistically non-significant reduction in absolute kidney weight was observed in males, respectively. The changes in organ weights were subscribed by the observations of histopathological changes in the liver and kidneys of the animals. Based on the short-comings in the design and/or reporting, no NOAEL could be determined and the study is considered to be supplementary.

An additional subacute oral toxicity study was performed by Mihail (1983), in which male and female rats received 0, 10, 100 and 1000 mg/kg bw/d pencycuron by gavage during a period of 14 days (10 animals/sex/dose). Half of the animals were autopsied at the end of the exposure period, the other half 4 weeks later. The main aim of the study was to investigate liver enzyme induction. Consequently, pathological examination was restricted to the determination of liver weights and clinical determinations to alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activities. No haematological examinations were performed. No changes in absolute and relative liver weights were observed nor other treatment related changes (body weight, behaviour, blood enzyme activities). An increase in the enzyme p-nitroanisole-O-demethylase in the liver from doses of 100 mg/kg bw/d onwards in male was found but not in female rats. . In view of the limited scope and duration of the study, it cannot be used to evaluate the subacute toxicity of pencycuron after oral administration.

Non-neoplastic effects observed in the chronic (see chapter 4.10) and effects on the parents in the reproductive toxicity studies (see chapter 4.11) were included in the assessment for STOT RE.

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

In a 21-days sub-acute dermal toxicity study (Diesing, 1992), pencycuron (purity 98.9%) was administered at 0, 250, 500, and 1000 mg/kg bw/d to 5 rabbits/sex/dose by the dermal route. The exposure was for 18 work days, 6 h/d, semi-occlusive for > 10% of the total body surface area. The study was performed in accordance with OECD 410 guideline. In the highest dose (1000 mg/kg bw/d) and the control group, an additional group of 5 animals/sex was incorporated as a satellite group and observed for a 2-week treatment-free period after the main study, to assess the possible recovery of delayed occurrence of any toxic effects.

No mortalities were found and no treatment-related effects in clinical signs, body weight gain, food consumption, and haematology.

Table 23 Results of a 21 days dermal toxicity study in rabbits:

Dose (mg/kg bw/d)	0		250		500		1000		dr
	m	f	m	f	m	f	m	f	
Clinical chemistry									
- triglyceride (liver)			i		ic		ic		m
Organ weights									
- liver				$dc^{a,r}$		dc^{r}		dc^{r}	
- kidneys				d^{r}		d^{r}		dc^{r}	f
Pathology									
Macroscopy	no treatment-related findings								
Microscopy									
Liver									
- pigmentation (brown) of hepatocytes	0/5	0/5	1/5	2/5	0/5	3/5	1/5	2/5	
Slight pigmentation in Kupffer cells	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
Testes									
- focal distension of tubules, unilateral	0/5		2/5		0/5		0/5		
- focal distension of tubules, bilateral	0/5		0/5		2/5		3/5		

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a,r absolute/relative organ weight
+ present in one/a few animals
++ present in most/all animals

Dermal exposure of rabbits to pencycuron at a concentration of 1000 mg/kg bw/d for 21 days resulted in effects on the liver and testes of males and on the liver and kidneys of females. Increased triglyceride concentrations in the liver of males were observed in all dose levels and this increase was statistically significant at concentrations of 500 and 1000 mg/kg bw/d. Since this effect was not observed in females and no correlation with other liver parameters was found it is not considered to be related to the treatment. Changes in the testes, that were observed at all dose levels, were also not considered to be treatment related, because no clear pattern was observed. Changes in liver and kidney weights of females were related to high organ weights in the main control group compared with the satellite control group and not to treatment. A fine granular brown pigment was seen in the haematoxylin and eosin stained sections of the liver of several rabbits in treated groups. The pigment was also visualised in control rabbits following Schmorl's stain, but it did not stain positive with Schmorl's stain or Perl's reagent. Overall, no clear pattern was observed for the abnormal colouration of the liver in both males and females and the presence of colouration depended on the type of staining used. Therefore, the NOAEL is set at 1000 mg/kg bw/d.

In another dermal 21-days study (Flucke and Gröning, 1981) 6 rabbits/sex/dose were treated with pencycuron (purity 99.4%) by the dermal route on 15 work days, 6 h/d (exposure area of 35 cm^2) at 0, 50, and 250 mg/kg bw/d. Because no guideline were available at the time the study was conducted, study is not in accordance with OECD 410. There are shortcomings in the performance of the study: 3 animals/sex/dose with intact skin and 3 animal/sex/dose with abraded skin were used, and therefore, the number of animals with intact skin per dose group was too low. The percentage of the total body surface area that was treated with the test substance was not indicated. The test substance was applied uncovered and during the exposure, the animals were immobilized and had no access to water and

food. Too few test concentrations were used. No local effects were observed. This study can not be used for classification and labeling purposes, no NOAEL was reported.

4.7.1.4 Repeated dose toxicity: other routes

No information available.

4.7.1.5 Human information

No information available.

4.7.1.6 Other relevant information.

This information is not available.

4.7.1.7 Summary and discussion of repeated dose toxicity.

Semi-chronic and chronic dietary toxicity studies in rat and mice and a 1-year chronic dietary toxicity study in dogs are available. In the oral toxicity studies, the main target for pencycuron seen in both rats and mice was the liver. Relative liver weights in male and female rats and mice were significantly increased this was accompanied by microscopic findings in the liver of males and females of abnormal distribution of chromatin in the nucleus and irregular nucleus size. These histopathological findings were clearly increased at the highest dose in both rat and mouse. In the 1-year study with dogs, no histopathological changes were observed in the liver and the differences in liver weight were small and not significant. In the 2-generation test, again an effect on the liver was observed (liver weight) in the parents (P0) and an increase in kidney weight in females (P0).

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE).

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

In two semi-chronic toxicity studies with rat and mouse, the liver was found to be the main target organ. The relative liver weights were increased significantly and this was accompanied by histopatological changes in the liver of abnormal distribution of chromatin in the nucleus and irregular nucleus size. However, according to the CLP criteria the effects should clearly indicate functional disturbance of morphological changes which are toxicological relevant. The liver is clearly the main target of pencycuron but the effects seen in both the 90-days toxicity studies with rat and mouse.

A sub-acute dermal toxicity study in rabbits is also available showing no local or systemic effects up to 1000 mg/kg bw/day. There is no information on repeated dose toxicity via inhalation.

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

According to the CLP classification criteria, the substance has to be classified for repeated dose toxicity, if significant or severe toxic effects, relevant for human health, are observed at dose levels ≤ 100 mg/kg bw/d in oral 90-days toxicity studies in rat. Based on the available 90-day study in rat, with a NOAEL of 120 mg/kg bw/d, in which significant increased relative liver weights were observed in males and females at the two highest dose groups (120 and 610 mg/kg bw/d) and minimal changes in the nucleus of the liver, these criteria are not fulfilled.

The effects observed in the 90-day toxicity study in mouse were the same as seen in the 90-day toxicity study in rat. Effect on the liver weight and minimal changes in the nucleus of the liver. The NOAEL for mouse was set at 65 mg/kg bw/d. The effects at a dose level of 264/315 mg/kg bw/day were limited to an increase in female liver weight and increases in some clinical chemistry parameters in males only. Based on this study no classification is warranted.

In the chronic toxicity studies in rats and mice, no effects at the cut-off dose for STOT RE 2 of 12.5 mg/kg bw/day were observed as the cut-off dose was above the NOAEL of 18 and 43 mg/kg bw/day for rats and mice, respectively. Also in the 2-generation test, the observed parental effects at the cut-off dose (100 mg/kg bw/day) were limited as the NOAEL was 32 mg/kg bw/day and at the LOAEL of 676 mg/kg bw/day only effects on liver and body weight were observed.

No guidance values are provided for the 1-year oral study in dogs. Assuming that the values for rats can also be applied to dogs including correction for the longer exposure periods, a guidance value of 25 mg/kg bw/day is used. The effects observed in relative liver weight were small and not statistically significant and not accompanied by histopathological changes. The NOAEL was set at 277 mg/kg bw/d. Based on this study no classification is warranted.

According to the EC classification criteria, the substance has to be classified for repeated dose toxicity, if significant or severe toxic effects, relevant for human health, are observed at dose levels ≤ 600 mg/kg bw/d in a dermal 28-days toxicity studies, using Haber's rule (600 mg/kg bw/day 28/21) the value would be ≤ 800 mg/kg bw/d for a dermal 21 days study. In the 21-day dermal toxicity study in rabbits no clear adverse effects were observed at 1000 mg/kg bw/d. The criteria are not fulfilled.

Comparison for the inhalation route is not possible due to absence of data.

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

The available oral toxicity studies do not show significant or sever toxic effects at dose levels requiring classification as STOT-RE.

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

Summary of the Dossier submitter's proposal

Subchronic dietary toxicity studies in rats and mice and a 1-year chronic dietary toxicity study in dogs were available. In the oral toxicity studies, the main target organ for pencycuron seen in both rats and mice was the liver. Relative liver weights in male and female rats and mice were significantly increased. This was accompanied by microscopic findings in the liver of males and females consisting of abnormal distribution of chromatin in the nucleus and irregular nucleus size. These histopathological findings were clearly increased at the highest dose in both rats and mice. In the 1-year study in dogs, no histopathological changes were observed in the liver and the differences in liver weight were small and not significant. In the 2-generation study, an effect on the liver (liver weight) in the parents (P0) and an increase in kidney weight in females (P0) were observed.

According to the CLP criteria, for classification as STOT RE, the effects should clearly indicate functional disturbance or morphological changes which are toxicologically relevant. The liver was clearly the main target organ, but the effects seen in both the 90-day toxicity studies in rats and mice were not considered of sufficient severity to justify classification.

A sub-acute dermal toxicity study in rabbits was also available showing no local or systemic effects up to 1000 mg/kg bw/d. There was no information on repeated dose toxicity via inhalation.

No classification was proposed by the DS for STOT RE.

Comments received during public consultation

Bayer had editorial comments on the description of the repeated dose toxicity study that were agreed by DS and were not considered to have an impact on classification decision.

Additional key elements

Available studies were conducted by oral (28-day, 90-day and 2-year studies in rats and mice and 1-year dog study) and dermal (21-day rabbit study) routes.

Subacute dermal studies (to be compared with the classification criteria range of 60-600 mg/kg bw/d for category 2 under CLP)

In a 21-day dermal study according to OECD TG 410 (Diesing, 1992), rabbits (n=5/dose/sex) were exposed to pencycuron at 250, 500 or 1000 mg/kg dw/d. A significant decrease in relative liver weight was observed in females at all doses. The changes were however not dose-related (details not shown) and were attributed to high liver weight in controls. A significant decrease in relative kidney weight was observed at the highest dose. An increase in triglyceride in liver was observed in males and was significant from 500 mg/kg bw/d. Microscopic examination revealed brown pigment in hepatocytes and focal distension of tubules in the testes in some animals of all exposed groups (see incidences in Table 1 below). No clear dose-response was observed for these effects and the relation to treatment was considered uncertain.

Table 1 – Main histopathological findings in the 21-day dermal rabbit study

Dose (mg/kg bw/d)	0	250	500	1000

	m	f	m	f	m	f	m	f
Liver - pigmentation (brown) of hepatocytes	0/5	0/5	1/5	2/5	0/5	3/5	1/5	2/5
Testes - focal distension of tubules, unilateral - focal distension of tubules, bilateral	0/5 0/5		2/5 0/5		0/5 2/5		0/5 3/5	

m=males; f= females

28-day oral studies (to be compared with the classification criteria range of 30-300 mg/kg bw/d for category 2 under CLP)

In a 28-day oral study partly in accordance with the OECD TG 407 (Inukai, 1978a), Sprague-Dawley rats were exposed through diet to 100, 1000 or 10000 mg/kg food (corresponding to 9.7/10, 100/102 or 1034/1 056 mg/kg bw/d in males/females, respectively). Deviations in the study design were limited reporting and use of clay (4%) as a vehicle at the highest test concentration, that may have altered the results. A significant decrease in body weight and body weight gain was observed in females from 10 mg/kg bw/d and in males at the highest dose. A significant increase in relative liver weight was reported in males from 100 mg/kg bw/d and at the highest dose in females. Moderate to marked epithelial proliferation of pelvis was observed in the kidney of one/a few males at 100 mg/kg bw/d (and not at the highest dose) and in one/a few females from 102 mg/kg bw/d (exact incidences not provided).

In another 28-day oral study partly in accordance with OECD TG 407 (Inukai, 1978b), mice were exposed through diet to 100, 1000 or 10000 mg/kg food (corresponding to 14.5/15.6, 154/165 or 1559/1758 mg/kg bw/d in males/females, respectively). Deviations were similar to the 28-day rat study. A significant increase in relative liver and brain weights were reported in males only from 154 mg/kg bw/d and a decrease in kidney weight was observed in males and females at the low and the high dose. Histopathological findings were reported in liver and kidney and are summarised in Table 2 below.

Table 2 – Histopathological findings in the 28-day oral mouse study

Dose (mg/kg bw/d)	**m** **0**	**f** **0**	**m** **14.5**	**f** **15.3**	**m** **154**	**f** **165**	**m** **1 559**	**f** **1 758**
Liver - foci of cell infiltration	0/10	1/10	0/10	3/10	0/10	3/10	1/10	2/10
- irregular nucleus size	0/10	1/10	1/10	1/10	3/10	0/10	3/10	2/10
Kidneys - epithelial proliferation of pelvis	2/10	1/10	1/10	3/10	2/10	2/10	4/10	3/10

m=males; f= females

90-day oral studies (to be compared with the classification criteria range of 10-100 mg/kg bw/d for category 2 under CLP)

In a 90-day study partly according to OECD TG 408 (Inukai, 1978a), Sprague-Dawley rats were exposed through diet to 80, 400, 2000 or 10000 mg/kg food (corresponding to 4.6/5.6, 24/28,

120/138 or 610/712 mg/kg bw/d in males/females, respectively). Deviations in the study design (absence of opthalmological examination of functional observational battery and limited reporting of histopathological findings) were not considered to affect the validity of the results of this study.
Body weight with respect to controls was slightly but significantly decreased in females from 138 mg/kg bw/d. Effects on organ weight consisted of an increase in adrenals weight in males (non-significant) and in females (significant) at the highest dose and a significant increase in relative liver weight in males from 24 mg/kg bw/d and in females from 138 mg/kg bw/d. Liver weight in males dosed with 24 mg/kg bw/d was significantly decreased but toxicological significance of this finding is unknown considering the increase observed at higher doses.
Histopathological findings were observed only in the liver and are summarised in Table 3 below. An incidence clearly related to treatment was identified only at the highest dose.

Table 3 – Main findings in the 90-day oral rat study

Dose (mg/kg bw/d)	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
	0	**0**	**4.6**	**5.6**	**24**	**28**	**120**	**138**	**610**	**712**
Body weight				-3%		-3%		-5%*		-6%*
Liver										
relative organ weight			-3%	-3%	-5%*	+3%	+6%*	+6%*	+11%*	+14%*
minimal change of nuclear polymorphism					1/10		2/10		5/10	6/10
abnormal distribution of chromatin in the nucleus				1/10	1/10	2/10	2/10		5/10	6/10
irregular nucleus in size									5/10	6/10

* statistically significant difference compared to control group
m=males; f= females

In another 90-day study partly in accordance with the OECD guideline 408 (Inukai, 1978b), mice were exposed through diet to 80, 400, 2000 or 10000 mg/kg food (corresponding to 9.7/13, 50/65, 264/315 or 1345/1552 mg/kg bw/d in males/females, respectively). Deviations were similar to those in the 90-day rat study.

No changes in body weight were observed. Effects on organ weight consisted of a significant increase in relative spleen weight in males from 264 mg/kg bw/d. A decrease in relative kidney weight was observed in females from 315 mg/kg bw/d. Relative liver weight was increased in males from 50 mg/kg bw/day as well as in females at the highest dose. A decrease in relative thymus and submaxillary glands weights were also observed in females from 13 mg/kg bw/d.
Histopathological findings were observed in the liver only and are summarised in table 4 below.

Table 4 – Main findings in the 90-day mouse study

	males				**females**			
Dose (mg/kg bw/d)	**9.7**	**50**	**264**	**1 354**	**13**	**65**	**315**	**1 552**
Liver								
relative organ weight	+0%	+2%	+2%	**+9%***	+5%	**+8%***	**+7%***	**+10%***
minimal change of polymorphism in the nucleus				4/10				5/10

abnormal distribution of chromatin in the nucleus				4/10			1/10	5/10
Thymus								
relative organ weight	+5.5%	+1%	-3%	-11%	**-19%***	**-12%***	**-21%***	-8%
Submxillary glands								
relative organ weight	-4%	-3%	-1%	+0%	**-13%***	-10%	**-25%***	**-22%***

* statistically significant difference compared to control group

A 90-day neurotoxicity study was performed in rats (n=12/sex/dose) according to OECD TG 424 (Schladt, 2006). Rats were exposed through diet to 500, 2500 or 15000 mg/kg food (corresponding to 35/51, 181/275 or 1168/1836 mg/kg bw/d in males/females, respectively).
Several gait abnormalities were observed during the study that were not observed in control animals. The effects were transient and occurred generally in the absence of a clear dose-response relationship (except for spatic gait at week 5) and in the absence of concomitant findings such as hindlimb foot splay and histopathology findings. They were considered to be of uncertain toxicological significance.

Increases in motor and locomotor activity were not dose-related and exceeded historical controls only at the highest dose. The effect was not considered treatment-related at lower doses.
Findings are summarised in Table 5 below.

Table 5 - Findings in the 90-day oral rat neurotoxicity study

Dose	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
(mg/kg bw/d)	**0**	**0**	**35**	**51**	**181**	**275**	**1168**	**1836**
Clinical signs[1]								
- Spastic gait (week 5-6)	0/12	0/12	2/12	2/12	3/12	3/12	5/12	9/12
- High-stepping gait (week 6-10)	0/12	0/12	2/12	4/12	3/12	1/12	1/12	6/12
- Uncoordinated gait (week 9-10)	0/12	0/12	1/12	1/12	2/12	0/12	3/12	0/12
Body weight gain					dc[2]		dc[2]	dc[2]
Food intake							ic	ic
Water intake						ic		ic
Functional observation battery[4]								
- Spastic gait (week 5)	0/12	0/12	1/12	2/12	3/12	3/12	5/12	9/12
- Stilted hindlimbs (week 8[5])	0/12	0/12	4/12	8/12*	3/12	3/12	2/12	5/12
- Walking on tiptoe (week 13)	0/12	0/12	1/12	0/12	0/12	0/12	0/12	0/12
Motor activity								
- Motor activity								ic[6]
- Locomotor activity								i[7]

dc/ic statistically significantly decreased/increased compared to the controls
1 Hair loss, a thin haircoat, an injury and/or a wound were also observed among animals.
2 During week 1 only, for the remaining study duration, body weight gain was similar for controls and dose groups.
3 Achieving statistical significance over Days 50-57 only.
4 No treatment-related findings were observed for other functional observations such as grip strength, foot splay and reflex testing.
5 Also observed in week 4 for one male and one female at 500 ppm and in week 13 for one male at 2500 ppm and one female at 15000 ppm.
6 Achieving statistical significance in week 2.
7 Achieving statistical significance in week 8.
* statistically significant ($p \leq 0.05$)

In a 2-generation study (Tauchi, 1990), Wistar rats were administered 50, 500 or 10000 mg/kg pencycuron in diet (approx. 3.2/4.6, 32/49 and 676/999 mg/kg bw/d in males and females, respectively). Body weight was significantly decreased in a transient way in females at 49 mg/kg bw/d and in males from 32 mg/kg bw/d and permanently in high dose females with respect to control. Effects on the weight of liver (relative increase from mid-dose), kidney (increased in all exposed F0 female groups) and spleen (decreased at high dose in F0 and F1) were observed. Magnitudes were not given. Microscopic finding were restricted to centrilobular swelling in the liver of high dose F0 and F1 animals.
Effects related to reproductive organs or developmental toxicity are described in the appropriate section below.

1-year oral study (to be compared with the classification criteria range of 2.5-25 mg/kg bw/d for category 2 under CLP)

In a 1-year study in accordance to OECD TG 409 (Bathe, 1983), Beagle dogs (n=6/sex/dose) were exposed through diet to 100, 1000 or 10000 mg/kg food (corresponding to 2.7, 31 or 277 mg/kg bw/d for males and females).

No significant change in body weight or food consumption were observed. A significant increase in absolute liver weight was observed in males only from 31 mg/kg bw/d, but the significance was not attained when relative weigths were considered. No histopathological changes were noted. Biochemical analysis revealed a significant increase in cytochrome P450 content of both male and female livers from 31 mg/kg bw/d. The main findings are summarised in Table 6.

Table 6 – main findings in the 1-year dog study

Dose (mg/kg bw/d)	**0**		**2.7**		**31**		**277**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Liver enzymes								
- Cytochrome P-450 (nmol/g)	12.1	12.1	12.4	12.5	15.9*	16.7*	30.4*	28.2*
Organ weights								
- liver (relative) (%)	2.8	3.0	2.5	2.7	2.9	3.0	3.1	3.2
- liver (absolute) (g)	216	214	214	216	247*	216	253*	237

* statistically significant difference with control group

2-year oral study (to be compared with the classification criteria range of 1.25-12.5 mg/kg bw/d for category 2 under CLP)

In a 2-year study according to OECD TG 453 (Shirasu, 1981a), F344 rats were exposed through diet to 50, 500 or 5000 mg/kg food (corresponding to 1.8/2.2, 18/22 or 186/229 mg/kg bw/d in males/females, respectively).

Body weight was significantly decreased in females at the highest dose. Total cholesterol was increased in females at high dose and total protein was increased in males at high dose. Liver weights were increased in males from 18 mg/kg bw/d at interim sacrifice and only in high-dose females at the terminal sacrifice. Microscopy examination revealed effects only at the highest dose. They consisted of increased incidences of lung bronchitis/bronchiolitis in males, hepatocellular hypertrophy and diffuse fatty changes in males and females, diffuse cortical fatty changes in the adrenals of males and renal nephrosis (males and females) and mineralization (females).
Neoplastic findings of this study are described under the carcinogenicity section.

In another 2-year study according to OECD TG 453 (Shirasu, 1981b), CD-1 mice were exposed through diet to 50, 500 or 5000 mg/kg food (corresponding to 4.4/4.2, 43/44 or 468/465 mg/kg bw/d in males/females, respectively). Body weight was significantly decreased in males at the highest dose. No other treatment-related changes were observed except a significantly increased incidence in diffuse hepatocellular swelling and degeneration in liver of high dose males.

Neoplastic findings of this study are described under the carcinogenicity section.

Assessment and comparison with the classification criteria

The main target organ for pencycuron is the liver with effects observed in rabbit by the dermal route and in rat, mouse and dog by the oral route. The effects generally consisted of an increase in liver weights (with males being more sensitive) that was accompanied in some cases by biochemical changes (increase in liver triglyceride content in rabbit or in cytochrome P450 content in dog) and/or by some histopathological changes. At the doses relevant for classification, the effects were as follows:

- In the 21-day rabbit dermal study - increase in triglyceride content in liver in males and brown pigment in hepatocytes in some animals of all exposed groups (from 250 mg/kg bw/d). No clear dose-response was observed for the latter effect and relation to treatment was considered uncertain.
- In the 28-day rat oral study - increase in relative liver weight (magnitude not given) at 100 mg/kg bw/d in males only without histopathological changes.
- In the 28-day mouse oral study - increase in relative liver weight (magnitude not given) in males at 154 mg/kg bw/d. Histopathological changes consisted of foci of cell infiltration and irregular nucleus size but no clear dose-response was observed.
- In both the 90-day rat and mouse oral studies - increase in relative liver weight (magnitude not given) in males only without histopathological changes (minimal change of nuclear polymorphism and abnormal chromatin distribution clearly elevated at doses above classification threshold only).
- In the rat 2-generation study - increase in relative liver weight at 32/49 mg/kg bw/d without histopathological changes.
- No effects in dogs (1-year oral study) or in the 2-year oral studies in rats and mice at doses relevant for classification.

Overall, although the magnitude of the increase in liver weight is not known, the absence of clear and/or severe histopathological findings at doses relevant for classification does not indicate an effect of sufficient severity to justify a classification.

Some effects were also identified in the kidney in rabbit by dermal route and in rat and mouse by oral route. At the doses relevant for classification, the effects were as follows:

- In the 28-day rat oral study - moderate to marked epithelial proliferation of pelvis of one/a few males at 100 mg/kg bw/d (and not at the highest dose) and in one/a few females from 102 mg/kg bw/d.
- In the 28-day mouse oral study - a decrease in relative kidney weight in males and females at the low and the high doses but not at mid-dose (14.5/15.6, 1559/1758, 154/165 mg/kg bw/d, respectively). The low incidence of epithelial proliferation of pelvis at the doses relevant for classification did not clearly indicate a treatment-related effect.
- In the rat 2-generation study, increase in relative kidney weight in F0 females from 4.6 mg/kg bw/d without histopathological changes.
- No effect in rabbit (21-day dermal study), dog (1-year oral study), rat (90-day and 2-year studies) or mouse (90-day and 2-year studies).
-

Overall, considering the absence of dose-response, the uncertainties of whether the effects were treatment related and the absence of effects at doses relevant for classification in studies with exposure longer than 28 days, kidney effects were not considered as sufficient to justify a classification.

Finally, isolated findings were observed in the following organs at doses relevant for classification:

- Focal distension in testicular tubules in the 21-day rabbit dermal study but incidences did not reveal a clear dose-response. In addition, this finding is considered to be relevant for the discussion of reproductive toxicity classification and not for STOT-RE.
- Increase in relative brain weight in males in the 28-day oral rat study but an effect on brain was not identified in the other studies available and was not accompanied by microscopic findings.
- Decrease in relative weight of thymus and submaxillary glands in the 90-day mouse oral study

but no effect was identified in these organs in the other studies available and the effects on weight were not accompanied by microscopic findings.

-

Overall, RAC agrees with the DS that these effects are not considered as sufficient to justify a classification as STOT RE.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 24: Summary table of relevant in vitro and in vivo mutagenicity studies with technical pencycuron (purity 98.5 – 99.2%).

Method	Results	Remarks	Reference
In vitro Ames test, Salmonella typhimurium	Negative (+/-, S9)	0 – 1000 µg/plate cytotoxicity and precipitation not reported	DAR, Inukai et al (1978c)
In vitro Ames test, Salmonella typhimurium	Negative (+ / -, S9)	10 – 5000 µg/plate cytotoxicity and precipitation not reported	DAR, Shirasu et al (1981)
In vitro Ames test, Salmonella typhimurium	Negative (+ / -, S9)	16 – 5000 µg/plate OECD 471	DAR, Herbold, (2008)
Bacillus subtilis Rec assay	Negative, - S9	0 – 300 µg/disc, solvent: DMSO	DAR, Inukai et al (1978d)
Bacillus subtilis Rec assay	Negative, - S9	0 – 5000 µg/disc, solvent DMSO	DAR, Shirasu (1981)
Bacillus E. coli Rec assay	Negative (+ / -, S9)	62.5 – 1000 µg/plate	DAR, Herbold (1981)
Saccharomyces cerevisiae (Yeast) Point mutation test	Negative (+ / -, S9)	625 – 10000 µg/plate	DAR, Brusick, (1982)
Saccharomyces cerevisiae (Yeast) Mitotic gene conversion assay	Negative (+ / -, S9)	625 – 10000 µg/plate	DAR, Brusick (1982)
Mammalian chromosome aberration study in human lymphocytes.	Negative (+ / -, S9)	0 – 100 µg/ml	DAR, Herbold (1986)
Mammalian chromosome aberration study in Chinese hamster lung cells	Negative (+ / -, S9)	$1{\cdot}10^{-3}$, $3.3{\cdot}10^{-4}$, $1{\cdot}10^{-4}$, $3.3{\cdot}10^{-5}$, $1{\cdot}10^{-5}$ M (+ S9) $1{\cdot}10^{-4}$, $3.3{\cdot}10^{-5}$, $1{\cdot}10^{-5}$, $3.3{\cdot}10^{-6}$ M (- S9)	DAR, Shirasu (1987)
UDS test in primary rat hepatocytes	Negative	Cytotoxicity test: 1.56, 3.13, 6.25, 12.5, 25, 50, 100 µg/ml UDS test: 5, 10, 15, 20, 30 and 40 µg/ml	DAR, (Lehn, 1990)
Mammalian gene mutation test (HGPRT) in chinese hamster ovary cells	Negative (+ / -, S9)	Preliminary cytotoxicity test: 10, 20, 40, 50, 60, 70, 80, 90 and 100 µg/ml (+/- S9) Mutation assay: 6.25, 12.5, 25, 50 and 100 µg/ml (+/- S9)	DAR (Brendler, 1991)
Mouse(BOR:NMRI) micronucleus test; chromosome aberration	Negative	0, 5000 (24 hrs), 5000 (48 hrs) and 5000 (72	DAR, Herbold (1990)

		hrs) mg/kg bw/d, administered intraperitoneally; once; sacrifice at 24, 48 and 72h after last dose	
Mouse (NMRI/ORIG), dominant lethal, chromosome aberration test	Negative	0, 2000 mg/kg bw/d, single oral administration of male mice.	DAR (Herbold, 1982)
Mouse (NMRI/W77), dominant lethal, chromosome aberration test	Negative	0, 2000 mg/kg bw/d, single oral administration of male mice	DAR (Herbold, 1983)

4.9.1 Non-human information

4.9.1.1 In vitro data

Three gene mutation studies with *Salmonella typhimurium* were available. Two studies (Inukai et al 1978c,1978d and Shirasu et al 1981) were carried out following a publication of Ames et. al because no guideline was available at that time. These tests were performed with technical pure pencycuron and although cytotoxicity and precipitation were not reported there are no doubts about the integrity of the studies and the results are considered fully acceptable.

The third study, an Ames study of Herbold (2008), performed according to OECD 471 using the direct plate and preincubation method. Pencycuron was assayed in duplicate in five *S. typhimuriom* strains (TA 1537, TA98, TA100, TA 1535, and TA102 at 16 – 5000 µg/plate both with and without metabolic activation. Dimethyl sulphoxide was used as a vehicle. Toxicity was observed at the highest test concentration with S9 and precipitation was observed at the two highest test concentrations. The test substance did not induce point mutations in *S. typhimurium*.

Pencycuron was tested in two *Bacillus susbtilis* Rec assays (Inukai et al 1978 and Shirasu et al 1981). Both studies were carried out according a publication of Kada et al (1972). The test were only carried out without metabolic activation. Cytotoxicity and precipitation were not reported and raw data were not available, although the were no doubts about the integrity of the studies and the results were in line with the other genotoxicity studies. The test substance did not induce DNA damage to *B. subtilis* strains in both tests.

In a third Rec assay with *E. coli* strains W3110/polA+ and (K12)p3478/polA$_1$- (Herbold, 1981), pencycuron was tested at 0 – 1000 µg/plate with and without metabolic activation following a publication of Rosenkrantz and Leifer (1980). For this study no cytotoxicity and precipitation were reported and raw data were not available. However, there were no doubts about the integrity of the study. The test substance did not induce DNA damage to *E. coli* strains.

A reverse mutation assay with yeast was performed at 1 – 10000 µg/plate both with and without S9 mix (Jagannath, 1981). This test is considered as not acceptable because the positive control with metabolic activation did not give a positive response, the part of the study without metabolic activation is acceptable and did not induce point mutations in *Saccharomyces cerevisiae*. The test was carried out following a publication and guidelines were not available at the time of performance.

Another reverse mutation assay for point mutation (Brusick, 1982) carried out with *Saccharomyces cerevisiae* strain D$_7$77 at 625 – 1000 µg/plate following a publication of Zimmerman (1975).

Cytotoxicity was not observed up to 1000 µg/plate and precipitation was not reported. The test substance did not induce point mutations in *Saccharomyces cerevisiae.*

A mitotic gene conversion assay (Brusick, 1982) was carried out in *Saccharomyces cerevisiae* strain $D_7$77 at 625 – 10000 µg/plate following a publication of Zimmerman (1975). OECD guideline were not available at the time of performance of the study and raw data were not reported. Cytotoxicity was not observed up to the highest concentration and precipitation was not reported. The test substance did not induce gene conversions in *Saccharomyces cerevisiae.*

Pencycuron was tested in a mammalian chromosome aberration test using human blood lymphocytes (Herbold, 1986) at 0 – 100 µg/ml with and without S9 following a publication of Moorhead (1960). The mitotic index was decreased at dose levels > 12 µg/ml. Precipitation was not reported and the method and results were not fully described. However, there are no doubts about the integrity of the study. The test substance did not induce chromosome aberrations in human blood lymphocytes.

A second mammalian chromosome aberration test with Chinese hamster lung cells (Shirasu, 1987) did not induce chromosome aberrations in Chinese hamster lung cells. Cytotoxicity was observed at dose levels > 3.3 x 10^{-4} M(+S9) and > 3.3 x 10^{-5} M (-S9). Although, there is not referred to a guideline or publication for the method used and the method and results are not fully described the outcome of the study is in line with other in vitro genotoxicity tests.

An UDS test carried out with primary rat hepatocytes (Lehn, 1990) did not follow OECD guideline. The cytotoxicity test was performed at 1.56 – 100 µg/ml and gave cytotoxicity at ≥ 40 µg/ml. The UDS test was carried out at 5 – 40 µg/ml and did not induce unscheduled DNA synthesis in primary rat hepatocytes. No repeat assay was performed.

Pencycuron was tested in a mammalian cell gene mutation test using chinese hamster ovary cells CHO-K_1-BH_4 at the HGPRT locus following a method of Hsie (1981), at the time of the study an OECD guideline was not yet available. A cytotoxicity study was performed with concentrations of 10 – 100 µg/ml with and without metabolic activation. Cytotoxicity was observed at dose levels ≥ 50 µg/ml with and without metabolic activation and precipitation was determined at > 60 µg/ml. The mutation assay was carried out at 6.25 – 100 µg/ml. The test substance did not induce gene mutations in CHO cells with and without metabolic activation.

4.9.1.2 In vivo data

Three in vivo studies are available.

In a micronucleus study, BOR:NMRI mice (Herbold, 1990), 5/sex/dose, were administered intraperitoneally once at 0 and 5000 mg/kg bw/d according to OECD 474 guideline. Bone marrow samples were taken 24, 48 and 72 hours after treatment. Toxicity was observed at dose level 5000 mg/kg bw/d, P/N ratio was changed and animals showed apathy, stretching of body, roughened fur, staggering gait and spasm. The test substance did not induce micronuclei in mouse bone marrow cells.

A dominant lethal study (Herbold, 1982) with mouse (NMRI/ORIG) followed the recommendations of the ad hoc chemogenetic committee (1978) because OECD guideline for this type of studies was not available at the time of performance. A single oral dose of 0 or 2000 mg/kg bw/d was administered to male mice. Toxicity was not observed. The test substance did not induce mutations in germ cells in mice.

The third study was a repeat study of the second with another mouse species (NMRI/W77) and followed also the recommendations of the ad hoc chemogenetic committee (1978). A single oral dose

of 0 or 2000 mg/kg bw/d was administered to male mice. Toxicity was not observed, the test substance did not induce mutations in germ cells in mice.

Herbold (1981) reported a mouse micronucleus test (5/sex/dose) at 0, 1000 and 2000 mg/kg bw/d. Test substance was orally administered with 2 applications at an interval of 24 hours and sacrifice at 6 hour after the last dose was given. The method and results are not fully described. OECD guideline was not available at the time of the study. The time between the last dose and sacrifice was too short and therefore this study is considered not acceptable and no conclusions were drawn.

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

Pencycuron was negative in all performed in-vitro and in-vivo mutagenicity studies.

Three studies in vitro studies on Salmonella typimurium (two point mutations and one point mutation/frame shift/ transition) were all negative. Three indicator tests: Rec-assays on the bacteria Bacillus subtilis and E. coli were negative. A point mutation test (reverse mutation assay) in yeast Saccharomyces cer.$D_7$77 was negative just like two mitotic gene conversion tests with Saccharomyces cer. $D_7$77. An in-vitro mammalian chromosome aberration test with human lymphocytes did not induce chromosome aberrations and an in-vitro mammalian chromosome aberration test with Chinese hamster lung cell was also negative. An UDS assay in rat hepatocytes did not induce unscheduled DNA synthesis and a mammalian gene mutation test at the HGPRT locus in Chinese hamster ovary cells was negative. The three in-vivo studies were all negative. The three studies were performed in mouse with different strains. One micronucleus study was carried out according to OECD guideline.

4.9.5 Comparison with criteria

Classification for inheritable genetic damage requires positive results in in vivo tests. Based on the negative results of the available in vitro and in vivo genotoxicity and mutagenicity studies, these criteria are not fulfilled.

4.9.6 Conclusions on classification and labelling.

According to CLP regulation EC 1272/2008, pencycuron did not need to classify for germ cell mutagenicity.

RAC evaluation of germ cell mutagenicity
Summary of the Dossier submitter's proposal Pencycuron was negative in all *in vitro* and *in vivo* mutagenicity studies. Three *in vitro* studies on *Salmonella typimurium* (two point mutation tests and one point mutation/ frame shift/ transition test) were all negative. Three indicator tests: Rec-assays on the bacteria *Bacillus subtilis* and *E. coli* were negative. A point mutation test (reverse mutation assay) and two mitotic gene conversion tests in yeast *Saccharomyces*

cer. D777 were negative. Pencycuron did not induce chromosome aberrations in an *in vitro* mammalian chromosome aberration test with human lymphocytes or in an *in vitro* mammalian chromosome aberration test with Chinese hamster lung cell. In an UDS assay in rat hepatocytes, induction of unscheduled DNA synthesis was not observed and a mammalian gene mutation test at the HGPRT locus in Chinese hamster ovary cells was negative. The three *in vivo* studies, a micronucleus study according to OECD TG 474, and two dominant lethal studies on different mouse strains following the recommendations of the ad hoc chemogenetic committee because OECD TG was not yet available, were all negative.

No classification was proposed by the DS for germ cell mutagenicity.

Comments received during public consultation

The DS agreed with Bayer's comments on the description of the data; however, the comments were not considered to have an impact on classification decision.

Additional key elements

In vitro, pencycuron did not induce gene mutations in bacteria. In mammalian cells, no effects were observed in the induction of gene mutations, chromosomal aberrations or unscheduled DNA synthesis.
In vivo, a micronucleus study according to OECD TG 474 was negative in the bone marrow of mice exposed intraperitoneally at the limit dose of 5000 mg/kg (single dose). Clinical signs and change in P/N ratio indicated that pencycuron was absorbed and reached the bone marrow.
Negative results were also obtained in two dominant lethal assays performed in the same laboratory in mice (two different strains) exposed to a single oral dose of 2000 mg/kg that did not induce signs of toxicity.

Assessment and comparison with the classification criteria

Negative results were obtained in all available mutagenicity tests performed, both *in vitro* and *in vivo* up to the limit dose.

RAC agrees with the DS that classification for mutagenicity is not warranted.

4.10 Carcinogenicity

Table 25: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
104 weeks oral diet, rat	NOAEL = 18 mg/kg bw/d (500 mg/kg food)	No increases of - neoplastic lesions. Liver effects: (hepatocellular hypertrophy, increased liver weight.	DAR Shirasu et al (1981)
104 weeks oral diet, mouse	NOAEL = 43 mg/kg bw/d (500 mg/kg food)	No increase of neoplastic lesions. Liver effects: diffuse hepatocellular swelling and degeneration	DAR Shirasu et al (1981)

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Pencycuron (purity 97.9%) was given by dietary admixture at dose levels 0, 50, 500, and 5000 mg/kg food (equal to 0, 1.8, 18, and 186 mg/kg bw/d for males and 0, 2.2, 22, and 229 mg/kg bw/d for females) to F344 rats (80/sex/dose and 8/sex/dose for interim kill) for 104 weeks (Shirasu et al., 1981a). The study was performed in accordance with OECD 453. Urine samples were taken in week 26, 51, 77, and 103. From eight animals per sex from each group blood was tapped for haematological and blood biochemistry examination in week 27, 52 (only males), 53 (only females) and 78. These examinations were performed on 10 animals per sex from each group in week 104. After blood sampling the animals were sacrificed for necropsy (including interim kills).

Table 26 with the results of a 104 weeks oral dietary carcinogenicity study in rats:

Dose	0		50		500		5000		dr
(mg/kg food)	m	f	m	f	m	f	m	f	
Mortality[1]	16/56	13/56	13/56	7/56	13/56	9/56	9/56	13/56	
Clinical signs	no treatment-related effects								
Body weight							d[2]	dc	
Food consumption	no treatment-related effects								
Ophthalmoscopy	not performed								
Haematology	no treatment-related effects								
Clinical chemistry									
- total cholesterol (wk 53, 78, 104)								ic	
- total protein (wk 27, 52, 78)							ic		
Urinalysis	no treatment-related findings								
Organ weights									
- liver (wk 26/27)					ic[a,r]		ic[a,r]		m
- liver (wk 52/53)							ic[a,r]	ic[r]	
- liver (wk 78)					ic[a,r]		ic[a,r]	ic[r]	m
- liver (wk 104)								ic[r]	
- kidneys (wk 104)								i[r],c[a]	
- adrenals (wk 26/27)					ic[a,r]		ic[a,r]		
Pathology									
macroscopy	no treatment-related effects								
microscopy									
neoplastic lesions									
Liver									
Hepatocellular adenoma	3/80	1/80	1/80	1/80	0/80	0/80	1/80	0/80	
Microscopy									
non-neoplastic lesions									
lung									
- bronchitis/ bronchiolitis	2/63		4/67		5/67		11/70		
liver									
- hepatocellular hypertrophy	0/79	0/80	0/80	2/80	2/79	0/80	22/79	19/80	
- nodular hepatocellular hypertrophy	8/79	8/80	11/80	2/80	14/79	13/80	27/79	35/80	
- diffuse hepatocellular fatty change	5/79	7/80	4/80	7/80	5/79	6/80	17/79	28/80	
adrenal									
- diffuse cortical fatty change							+		
kidneys									
- nephrosis							+	++	
- mineralization								++	

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute organ weight, relative organ weights were not indicated
\+ present in a few animals more than in the control groups
++ present in many animals more than in the control groups

[1] Excluding animals used for interim kills
[2] The significant decrease was only seen in week 1 to 16 and was not found thereafter.

No statistically increased mortality occurred. A reduced body weight was observed in the highest dose level (5000 mg/kg food) of both sexes: in males body weight decreased in week 1 – 16 with ≤ 3% in females a statistically significant decrease was noted during the entire study with > 10%. As this reduction could not be attributed to changed food consumption, as this was normal as compared to control group, it was considered to be related to treatment. In females, a significant increase of total cholesterol was observed in the high dose group (5000 mg/kg food). In both males and females, some kidney effects (nephrosis and mineralization) were observed at the highest dose. The liver weights in males and females were increased at the highest dose level of 5000 mg/kg food (see table 26).

Table 27: Changes in absolute and relative liver weight:

Liver weight in (% of control values)								
week	27	52	78	104	26	53	78	104
	Males				females			
dose	Mean absolute liver weight							
50	+3	-1	+5	-2	-6	+3	-2	+3
500	+11**	+4	+15**	+3	-4	-2	+3	-2
5000	+24***	+19***	+22***	0	+5	+6	+3	+3
	Mean relative liver weight							
50	+3	+1	+2	-3	-9*	-1	-5	+3
500	+9***	+4	+11*	+2	-4	-2	+4	-3
5000	+24***	+21***	+21***	+3	+10*	+12***	+14**	+15***
*, **, *** significantly different from control at 5, 1, 0.1% level								

Liver weight increase was also observed in males at 500 mg/kg food in week 27 and 78, but occurred not consistently throughout the study period and were therefore not considered toxicologically relevant. The increased liver weights at 5000 mg/kg food were associated with histopathological lesions (hepatocullular hypertrophy, nodular hepatocellular hyperplasia and diffuse hepatocellular fatty change) in the liver. An increased incidence of these non-neoplastic lesions in the liver was clearly noted at 5000 mg/kg food. At 500 mg/kg food, the observed incidence in nodular hepatocellular hyperplasia was considered comparable to controls. In males, a dose related increase in bronchitis/brochiolitis was observed, which clearly reached statistical significance in the highest dose group. The authors of the report (Shirasu *et al.*) did not comment upon this adverse effect. As these lung effects were restricted to males, even at the highest dose, and were not observed in any other study, they may be a chance finding. Based on the observed liver effects at 5000 mg/kg food, the NOAEL is set at 500 mg/kg food (18 mg/kg bw/d). Pencycuron did not show carcinogenic potential in rats.

In a second combined chronic toxicity / carcinogenicity study, pencycuron (purity 97.9%) was administered by diet to CRJ : CD-1 mouse (80/sex/dose (including 10 sex/dose for interim kill) at 0, 50, 500, and 5000 mg/kg food (equal to 0, 4.4, 43, and 468 mg/kg bw/d for males and 0, 4.2, 44, and 465 mg/kg bw/d for females) for 104 weeks (Shirasu et al, 1981b). The study was carried out according to OECD 453 guideline, except the duration of the study, this should be 18 months for mice. However, since at 18 months survival in all experimental groups was > 50%, this deviation is considered acceptable. Urinalysis was performed in 10 animals/sex/dose in week 51 and 103 of

treatment. Blood samples of ten animals of each sex from each dose group after 52 weeks and at the termination of treatment were taken for haematological and blood biochemical analysis. Pathological examinations were performed on 10 animals per dose group and sex after 52 weeks and on all surviving animals at the termination of treatment.

Table 28 Results of a 104 weeks repeated dietary toxicity study in mice

Dose	0		50		500		5000		dr
(mg/kg food)	m	f	m	f	m	f	m	f	
Mortality	50/70	51/70	57/70	46/70	56/70	54/70	52/70	44/70	
Clinical signs	no treatment-related effects								
Body weight							dc		
Food consumption	no treatment-related effects								
Ophthalmoscopy	not performed								
Haematology	no treatment-related effects								
Clinical chemistry	no treatment-related effects								
Urinalysis	no treatment-related effects								
Organ weights	no treatment-related effects								
Pathology									
macroscopy	no treatment-related effects								
microscopy									
liver									
- diffuse hepatocellular swelling and degeneration					i		ic	i	m
neoplastic lesions									
Hepatocellular adenoma	21/80	2/79	17/80	3/80	14/80	2/80	9/80	2/80	
Hemangioma	3/80	1/79	0/80	2/80	2/80	0/80	1/80	2/80	
Hepatocellular adenocarcinoma	4/80	0/79	2/80	1/80	4/80	0/80	2/80	0/80	
Hepatoblastoma	1/80		2/80		2/80		0/80		
Neoplastic nodule (not in section)	2/80		1/80		0/80		0/80		

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

Chronic oral administration of pencycuron during 104 weeks to mice significantly decreased the body weight gain of males at a dose of 5000 mg/kg food (stat. significant decreases in bw during the largest part of the study; decreases >10% in first half of study).

At 5000 mg/kg food, the incidence of diffuse hepatocellular swelling and degeneration was significantly increased in males, but not in females. Although this change was also observed in the control mice, the degree and extent of the lesions were more severe in males at 5000 mg/kg food than those in controls. The incidence of diffuse hepatocellular swelling and degeneration was as follows:

Table 29: Incidences of diffuse hepatocellular swelling and degeneration (number affected/number investigated)

Dose (ppm)	0	50	500	5,000
Male	2/80	1/80	6/80	12/80 *
	(+)	(+)	(+ - ++)	(+ - +++)
Female	1/79	2/80	1/80	4/80
	(++)	(+ - ++)	(+ - ++)	(+ - ++)

* $p < 0.05$ (chi^2 test), (+) = slight, (++) = moderate, (+++) = severe

Since only at 5000 mg/kg food severe diffuse hepatocellular swelling and degenation was noted in males, and the increase in slight to moderate liver changes also occurred in males and females in the control group, the observed changes at 5000 mg/kg food were considered toxicologically relevant. The increased incidence of diffuse hepatocellular swelling and degeneration found in males of the 500 mg/kg food group was considered marginal. No further treatment-related effects were observed. Based on the effects seen in the liver of males at a dose of 5000 mg/kg and the dose-related increase of these effects, the NOAEL was set at 500 mg/kg food (equal to 43 mg/kg bw/d). Pencycuron did not show carcinogenic potential in mice.

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

No information available.

4.10.3 Other relevant information

No information available.

4.10.4 Summary and discussion of carcinogenicity.

Two oral chronic toxicity and carcinogenicity studies are available, One with rats and the other with mice. In both studies animals were treated by diet for 104 weeks at 0, 50, 500, and 5000 mg/kg food. The liver was observed as the main target organ in rat. At the highest dose level hepatocellular hypertrophy, nodular hepatocellular hyperplasia and diffuse hepatocellular fatty change was noted combined with increased liver weight (relative and absolute). There was no increase in neoplastic lesions, therefore the substance did not show carcinogenic potential in rats. In the study with mouse, the liver was also the main target, at the highest dose level of 5000 mg/kg food diffuse hepatocellular swelling and degeneration of the liver in males was observed with was significantly increased compared to control. The body weight gain of males at 5000 mg/kg food was significantly decreased. No increases of neoplastic lesions was found.

4.10.5 Comparison with criteria

Classification for carcinogenicity requires an increased incidence of neoplasms due to exposure to the substance. Pencycuron did not show carcinogenic potential in two combined chronic toxicity / carcinogenicity studies. Therefore, classification for carcinogenicity is not warranted.

4.10.6 Conclusions on classification and labelling

Classification for carcinogenicity is not needed.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

Two oral chronic toxicity and carcinogenicity studies were available, one in rats and one in mice. In both studies, pencycuron was administrated orally for 104 weeks at doses of 0, 50, 500, and 5000 mg/kg food. The main target organ in rats was the liver . At the highest dose, hepatocellular hypertrophy, nodular hepatocellular hyperplasia and diffuse hepatocellular fatty change were noted and were associated with increased liver weight (relative and absolute). There was no increase in neoplastic lesions, therefore the substance did not show carcinogenic potential in rats. In the mouse study, the liver was also the main target organ. At the highest dose of 5000 mg/kg food, diffuse hepatocellular swelling and degeneration of the liver in males were observed that were significantly increased compared to controls. The body weight gain of males at 5000 mg/kg food was significantly decreased. No increases of neoplastic lesions were found.
No classification was proposed by the DS for carcinogenicity.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

No evidence of carcinogenicity was reported in the rat and mouse 2-year dietary studies. In particular, no elevation (significant or not) of the incidences in liver tumours (main target organ) were observed in either rat or mouse.
RAC agrees with DS that classification for carcinogenicity is not warranted.

4.11 Toxicity for reproduction

Table 30: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
2 generation reproduction toxicity study with rat	NOAEL parental = 32 mg/kg bw/d. NOAEL developmental = 32 mg/kg bw/d NOAEL reproduction ≥ 676 mg/kg bw/d	Decreased body weight, increased liver weight. Decreased pup body weight. none	DAR Tauchi, 1990
Developmental oral gavage study, rat, dosing during gestation from day 6 to 20.	NOAEL maternal ≥ 1000 mg/kg bw/d NOAEL development ≥ 1000 mg/kg bw/d	Slightly increased incidences in soft tissue variations and skeletal variations were noted, which remained within historical control ranges.	DAR, Langrand-Lercher (2008)
Developmental oral gavage study, rabbit, dosing during gestation from day 6 - 18.	NOAEL maternal ≥ 2000 mg/kg bw/d NOAEL development ≥ 2000 mg/kg bw/d.	No teratogenic potential	DAR, Becker, 1983

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Pencycuron (purity 98.4 – 98.1%) was given by dietary admixture at concentrations of 0, 50, 500, and 10000 mg/kg food (equal to 0, 3.2, 32, and 676 mg/kg bw/d for males and 0, 4.6, 49, and 999 mg/kg bw/d for females) to groups of 27 Wistar rats/sex/dose continuously through the study (F0 and F1) (Tauchi, 1990). The study was performed in accordance with OECD 416 except the histopathological examination of the pups and the organ weight of brain, pituitary, thyroid and adrenal glands, prostate and seminal vesicles. The F0 and F1 generation were both mated twice.

.

Table 31 with the results of a dietary fertility study in rats

Dose (mg/kg food)	0		50		500		10000		dr
	m	f	m	f	m	f	m	f	
F0 animals									
Mortality	0/27	0/27	0/27	0/27	0/27	0/27	0/27	1* /27	
Clinical signs	no treatment related findings								
Body weight					dc[1]	dc[1]	dc[1]	Dc	
Food consumption					dc[1]		dc[1]	dc[1]	
Mating/fertility/gestation	no treatment related findings								
Organ weight									
- liver					ic[a,r]	ic[r]	ic[a,r]	ic[a,r]	m,f
- kidney				ic[a,r]		ic[a,r]		ic[r]	
- spleen							dc[a,r]	dc[a,r]	
- testis							dc[a]		
Testis weights (mg)									
absolute +SD	3191 ± 187		3157 ± 176		3151 ± 327		3090 ± 172		
Relative +SD	560 ± 50		550 ± 35		549 ± 60		548 ± 41		
- ovary						ic[a,r]		ic[a,r]	
Ovary weights (mg)									
absolute +SD		109 ± 19		110 ± 18		129 ± 35		124 ± 21	
Relative +SD		32 ± 6		32 ± 6		39 ± 10		39 ± 6	
Pathology									
macroscopy	no treatment related findings								
microscopy									
liver (centrilob. swelling)							ic	ic	
F1 pups									
Litter size, first parity	12.1 ± 2.8		11.6 ± 3.2		12.7 ± 2.4		12.8 ± 2.0		
Litter size second parity	12.6 ± 2.8		11.4 ±3.8		11.6 ± 2.8		12.2 ± 3.5		
Survival index, first parity, mean [%]									
Day 0 – 4	91.0		88.9		95.1		87.4		
Day 4 - 21	93.1		95.7		93.1		95.5		
Survival index, second parity, mean [%]									
Day 0 – 4	85.3		71.5		95.2		91.8		
Day 4 - 21	82.5		68.8		82.3		82.4		
Sex ratio (male/female)									
first parity	0.93		0.85		0.93		1.18		
second parity	0.98		1.04		0.85		1.23		
Body weight							dc	dc	
Pathology									
macroscopy	no treatment related findings								
F1 animals									

Dose (mg/kg food)	0		50		500		10000		dr
	m	f	m	f	m	f	m	f	
Mortality	0/27	0/27	0/27	0/27	0/27	0/27	0/27	0/27	
Clinical signs	no treatment related findings								
Body weight							dc[1]	dc[1]	
Food consumption							dc[1]	dc[1]	
Mating/fertility/gestation	no treatment related findings								
Organ weight									
- liver						ic[a,r]	ic [a,r]	ic[a,r]	f[a,r]
- kidney			ic[a]						
- spleen			ic[a,r]				dc[a,r]	dc[a,r]	
Pathology									
macroscopy	no treatment related findings								
microscopy									
-liver (centrilob. swelling)							ic	ic	
F2 pups									
Litter size, first parity	10.3 ± 3.2		10.7 ± 3.1		12.4 ± 2.9 #		12.1 ± 3.9		
Litter size second parity	12.5 ± 3.9		11.1 ± 4.1		11.8 ± 3.7		12.1 ± 4.1		
Survival index, first parity, mean [%]									
Day 0 – 4	98.0		92.7 #		88.8		89.6 #		
Day 4 - 21	93.5		97.6		96.5		85.5		
Survival index, second parity, mean [%]									
Day 0 – 4	95.0		95.7		98.6		97.1		
Day 4 - 21	92.0		96.0		95.6		93.3		
Sex ratio (male/female)									
first parity	1.05		1.07		0.98		0.98		
second parity	0.90		0.93		0.97		1.02		
Body weight					dc	dc	dc	dc	
Pathology									
macroscopy	no treatment related findings								

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a,r absolute/relative organ weight
\+ present in one/a few animals
++ present in most/all animals
* one dam died of foreign body pneumonia due to the inhalation of food and nesting material
[1] significant effect over a distinct, but not the whole, treatment period
\# significant different from control ($p<0.05$)

Table 32 body weight changes F0 animals

Dose (mg/kg food)	0		50		500		10000		dr
	m	f	m	f	m	f	m	f	
Body weight change [% compared to control and day 0 weight] significant values are reported only									
Week 1									
Week 2					-10	-11	-25	-30	
Week 3					-6	-10	-16	-18	
Weel 4					-3	-8	-12	-15	
Week 5					-2		-9	-12	
Week 6					-1		-6	-11	
Week 7					-1		-5	-10	
Week 8								-8	
Week 9								-9	
Week 10								-8	
Week 11								-9	
First parity pregnancy								-9	
Day 0									
Day 7								-8	
Day 14								-10	
Day 21								-11	
Lactation								-11	
Day 0									
Day 4								-15	
Day 7								-18	
Day 14								-17	
Day 21								-14	
Second parity pregnancy								-14	
Day 0									
Day 7								-8	
Day 14								-7	
Day 21								-8	
Lactation								-8	
Day 0									
Day 4								-9	
Day 7								-12	
Day14								-12	
Day 21								-9	
								-10	

Table 33 body weight changes F1 adults

Dose (mg/kg food)	0		50		500		10000		dr
	m	f	m	f	m	f	m	f	
Body weight change [% compared to control and day 0 weight] significant values are reported only									
Week 1									
Week 2							-9	-13	
Week 3							-13	-8	
Weel 4							+2	-5	
Week 5							-13	-2	
Week 6							-11	-2	
Week 7							-7	-1	
Week 8							-8		
Week 9							-8		
Week 10							-5		
Week 11							-5		
							-4		

Table 34 body weight changes F1a pups

Dose (mg/kg food)	0		50		500		10000		dr
	m	f	m	f	m	f	m	f	
Body weight change [% compared to. control] significant values are reported only									
Days after delivery							-22	-19	
Day 4 (pre-culling) – 0							-4	-7	
Day 7- 4 post-culling							-12	-11	
Day 14- - 4 post-culling							-22	-21	
Day 21 - - 4 post-culling									

Table 35 body weight changes F1b pups

Dose (mg/kg food)	0		50		500		10000		dr
	m	f	m	f	m	f	m	f	
Body weight change [% compared to control] significant values are reported only									
Days after delivery									
Day 4 (pre-culling) – 0									
Day 7- 4 post-culling									
Day 14- 4 post-culling							-15		
Day 21 - 4 post-culling									

Table 36 body weight changes F2a pups

Dose (mg/kg food)	0		50		500		10000		dr
	m	f	m	f	m	f	m	f	
Body weight change [%compared to control] significant values are reported only									
Days after delivery					-23	-23	-35	-35	
Day 4 (pre-culling) – 0					-10	-8	-12	-19	
Day 7- 4 post-culling							-11	-15	
Day 14- 4 post-culling					-12	-8	-24	-28	
Day 21 - 4 post-culling									

Table 37 body weight changes F2b pups

Dose (mg/kg food)	0		50		500		10000		dr
	m	f	m	f	m	f	m	f	
Body weight change [% compared to control] significant values are reported only									
Days after delivery					-6	-24	-12	-18	
Day 4 (pre-culling) – 0					-10	-14	-14	-22	
Day 7- 4 post-culling					-11	-7	-17	-19	
Day 14- 4 post-culling					-12	-9	-23	-26	
Day 21 - 4 post-culling									

Table 38: Kidney weights in F0 females

Dose (mg/kg food)	0	50	500	10000
Absolute (mg±SD)	2483± 63	2732±200***	2611±201*	2514±111
Relative (mg/100 g±SD)	722±51	778±66**	794±57***	786±39***

*,**,*** significantly different from control value at P<0.05 (*), P<0.01(**), P<0.001(***)

All relevant effects regarding parental toxicity were found at the dosing levels of 500 and 10000 mg/kg food. In the highest dose group food intake and body weight of the parental groups were decreased. The liver was in both generations (F0 and F1) and sexes the target organ on both microscopical and macroscopical level. In the F0 and F1 animals treated with 10000 mg/kg food a significantly increased absolute and relative liver weight and an increased incidence of centrilobular hepatocellular swelling were found. In the 500 mg/kg dosing group the absolute and relative liver weight in F0 males and females was slightly increased. These changes were not accompanied with histopathological findings in the liver and considered not toxicologically relevant. A decrease of absolute and relative spleen weight was also considered as not toxicologically relevant due to inconsistency of the findings and no dose-relationship was found.

No microscopically abnormalities were found in the F0 animals in both the testis and ovary.

Developmental effects were also found in the 500 and 10000 mg/kg food dosing groups. The body weight of pups was decreased in the highest dosing groups for both generations and at the 500 mg/kg dosing group for the F2-pups. However, body weights of F2a and F2b pups were within the normal range, but were lower than the unusually high control pup weights. These were mainly caused by smaller litter sizes, complete loss of potentially weak pups, and/or selection of the smallest pups for culling in the control groups. Observed changes in pup body weight at 500 mg/kg food were therefore not considered toxicologically relevant.

Pencycuron did not influence the reproductive performance of the animals. Based on, respectively, decreased body weight of the pups, and on decreased body weight and increased liver weight in the parents at the next higher dose, a dose of 500 mg/kg food pencycuron in the normal diet (ca. 32 mg/kg bw/d) is considered to be the NOAEL for both developmental and parental effects.

Another 2- generation study in rats is mentioned in the DAR. However, this study is not described in the DAR because the NOAEL for parental and developmental effects is set at 1000 mg/kg food (58 mg/kg bw/d), based on a slight decreased body weight gain at F0 and F1 males and an increase in liver and kidney weights (F1 and F2) observed in the highest dose group. No adverse treatment related reproductive effects were observed in any of the dose groups. The study was not elaborately evaluated in the DAR, because the lowest level tested in this study is higher than the NOAEL levels as derived in Study 1 (Tauchi, 1990) and because the results are consistent with those obtained in Study 1.

4.11.1.2 Human information

No information available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

In a study of Langrand-Lercher (2008), four groups of 23 pregnant female rats (Sprague-Dawley) received pencycuron (purity 98.7%) by gavage in a solution with 0.5% aqueous methylcellulose at 0, 40, 200, and 1000 mg/kg bw/d from day 6 to 20 of gestation. Necropsy on day 21 of gestation.

The study was performed in accordance with OECD 414.

Table 39: Results of developmental toxicity study with pencycuron in rats.

Dose (mg/kg bw/day)	0	40	200	1000	dr
Maternal effects					
Mortality	1/23	0/23	0/23	0/23	
Clinical signs		No treatment-related findings			
Pregnant animals	21/23	20/23	23/23	23/23	
Body weight (gain)		No treatment-related findings			
Gravid uterus weight		No treatment-related findings			
Food consumption		No treatment-related findings			
Liver weights		No treatment-related findings			
Pathology					
macroscopy		No treatment-related findings			
Litter response					
Number of dams examined	21	20	23	23	
Copora lutea	339	351	411	402	
Live foetuses	284	284	334	333	
Foetal weight		No treatment-related findings			
Early resportions	18	18	27	22	
Pre implantation loss per litter (%)	10.6	13.1	10.8	11.4	
Post implantation loss per litter (%)	6.5	5.5	7.3	6.6	
Sex ratio		No treatment-related findings			
Examination of the foetuses					
External observations		No treatment-related findings			
Skeletal findings					
- incomplete ossification hyoid centrum (% of litters affected)	4.8	10.0	13.0	13.0	
- incomplete ossification hyoid centrum (% of fetuses affected)	1.4	1.4	1.8	2.9	
- unossified 7th cervival centrum (% of litters affected)	14.3	0.0	13.0	17.4	
- unossified 7th cervival centrum (% of fetusses affected)	4.8	0.0	2.9	2.3	
- discontinuous costal cartilage (% of litters affected)	33.3	35.0	21.7	39.1	
- discontinuous costal cartilage (% of fetuses affected)	4.8	5.5	4.7	5.8	
- incomplete ossification 5th metacarpals (% of litters affected)	4.8	0.0	4.3	8.7	
- incomplete ossification 5th metacarpals (% of fetuses affected)	1.4	0.0	1.2	1.7	
- less than 9 sacrocaudal vertebrae ossified (% of litters affected)	4.8	5.0	0.0	13.0	
- less than 9 sacrocaudal vertebrae ossified (% of litters affected)	2.0	0.7	0.0	2.3	
Visceral findings					
- renal pelvis dilatation (%of litters affected)	0	0	0	8.7	
- renal pelvis dilatation (% of fetuses affected)	0	0	0	1.2	
- dilated ureters (% of litters affected)	42.9	40.0	47.8	52.2	
- dilated ureter (% of fetuses affected)	11.7	11.6	12.9	11.2	

The findings: a slightly increased incidence in the occurrence of renal pelvis dilatation in the high dose foetuses, a slight increase in occurrence of dilated ureter at 200 mg/kg bw/d and at 1000 mg/kg bw/d were only slightly outside (but comparable to recent study data) or within the historical control range. Historical control range (HCD) for renal pelvis dilatation in the litters and fetuses: 0.0 – 8.3% and 0.0 – 1,2%, respectively. The HCD for the occurrence of dilated ureter in the litters and foetuses: 30.4 – 75.0% and 7.4 – 29 7%, respectively. Skeletal examinations showed slightly increased incidence of unossified incidences of variations at 1000 mg/kg bw/day.

A slight non-dose related increased incidence of unossified 7th cervical centra was within the historical control range (HCD for litters: 0.0 – 41.7% and for foetuses: 0.0 – 12.2%). Also the non-dose related increased incidence of incomplete ossified 5th metacarpals was within the historical control range (HCD for litters: 0.0 – 9.1% and for foetuses: 0.0 – 3.2%), which also applies for the increased incidence of delayed ossification of sacraudal vertebrae(HCD for litters: 0.0 – 20.0% and for foetuses: 0.0 – 3.7%).

The incidence of incomplete ossification of the hyoid centrum was higher in all treatment groups when compared to controls based on % litters affected and at 200 and 1000 mg/kg bw/day based on % fetuses affected. Values were only slightly outside the historical control ranges (HCD for litters: 0.0 – 12.5% and for foetuses: 0.0 – 1.6%). The incidence of one discontinuous costal cartilage was slightly outside the historical control range at the litter level, but not at the fetal level (HCD for litters: 9.1 – 38.1% and for foetuses: 1.3 – 8.4%).

The NOAEL for maternal toxicity in this study is ≥ 1000 mg/kg bw/day. Although slightly increased incidences in soft tissue variations and skeletal variations were noted, since these findings remained within or only slightly outside the historical control ranges and considered not to be adverse, the NOAEL for developmental toxicity was set at ≥ 1000 mg/kg bw/day. Since no treatment–related irreversible structural effects were reported, the NOAEL for teratogenic effects is set at ≥ 1000 mg/kg bw/day, the highest dose tested.

In a second study of Becker (1983), pencycuron (purity 99.2%) was orally administered to 16 female rabbits per dose group at 0, 200, 600, and 2000 mg/kg bw/d by gavage from day 6 – 18 after mating.

The study was performed in accordance with OECD 414 except the macroscopic examination of the dams, which was not carried out. The dams were killed on day 28 of pregnancy and the foetuses removed by caesarean section.

Table 40 with the results of developmental toxicity study with pencycuron in rabbits.

Dose (mg/kg bw/day)	0	200	600	2000	dr
Maternal effects					
Mortality	0/16	0/16	0/16	1/16	
Clinical signs	no treatment related findings				
Pregnant animals	15/16	14/16	16/16	16/16	
Resorption	no treatment related findings				
Body weight gain	no treatment related findings				
Food consumption	no treatment related findings				
Organ weight					
- uterus	no treatment related findings				
Pathology					
macroscopy	not performed				
Litter response					
Live fetuses	no treatment related findings				
Fetal weight					
- on litter base				ic	
- on individual base	no treatment related findings				
Sex ratio	no treatment related findings				
Examination of the fetuses					
External observations	no treatment related findings				
Skeletal findings	no treatment related findings				
Visceral findings	no treatment related findings				

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

The only effect of pencycuron found in this teratogenicity study in rabbits was an increased foetal weight in the highest dosing group. This effect was only significant if based on litter, not if based on individual animals. Therefore, it is not considered as relevant. No treatment related maternal effects were found. Based on these findings both the NOAEL (maternal) and the NOAEL(developmental) are set at the highest concentration tested, 2000 mg/kg bw/day. No indication of a teratogenic potential of pencycuron in rabbits was found.

A third teratogenicity study is described in the DAR (Inukai and Iyatomi 1978) with pencycuron (purity 97.8%) with rats. 25 – 28 females were dosed by gavage at 0, 40, 200, and 1000 mg/kg bw/d from day 7 to 14 of gestation. This study is not acceptable.

This study has some shortcomings: a remarkable amount of animals from the control and dosing groups died during the experiment (mortality rates of 8% - 28%). The offspring was also affected, in the highest dose group almost all foetuses of two litters (in total 27 of 28 foetuses) were found dead. The two litters were excluded from the study and not further taken into account. This is questionable, the mortality could be based on faulty handling and stress from dosing or it would indicate that the test compound causes toxicity. After dosing the pregnant rats in the control and all treatment groups were sedated for 3 – 4 hours. This is an indication that the quality of the study performance regarding practical performance and animal handling is not acceptable.

In addition, the dosing period, which was short and did not cover the period of at least the implantation until the day prior to the scheduled caesarean section as requested in the guideline. The dose administration was performed until day 14 and not until the day prior to the termination of the study at day 20 of gestation, which is the practice. Relevant data like maternal food consumption, organ weight and pathology were not examined. Furthermore, it is unclear whether the dose levels were selected based on a range-finding study. The discrimination of foetal deaths from embryonal deaths was based on the visibility of tail and extremities, which is a rather unusual approach.

4.11.2.2 Human information

No information available.

4.11.3 Other relevant information

No information available.

4.11.4 Summary and discussion of reproductive toxicity

One extensive described two-generation study in rats is available, in which no indication for reproduction toxicity was found. In the 21-day dermal repeated dose toxicity study, an increase in focal distension of the tubules of the testes was observed at the highest dose level in rats. However, no clear pattern was observed. In addition, such effects were not observed in other repeated dose studies nor in the 2-generation study.

Two developmental /teratogenicity studies are available, one performed with rats and the other with rabbits. In both studies no treatment-related effects on maternal or litter parameters including external fetal observations were found. No indication of a teratogenic potential of pencycuron in rats and rabbits was observed. In the 2-generations some developmental effects were observed at the highest dose level in the form of reduced body weights. However, the maternal body weight was also reduced at this dose level.

4.11.5 Comparison with criteria

Classification for effects on sexual function and fertility require evidence showing an adverse effect on sexual function and fertility that is not considered to be a secondary non-specific consequence of other toxic effects. No effects on sexual function and fertility were observed in the available repeated dose and 2-generation studies. The criteria are not fulfilled.

Classification for effects on development require evidence showing an adverse effect on development that is not considered to be a secondary non-specific consequence of other toxic effects. Since there is no indication of teratogenicity or developmental effects, the criteria are not fulfilled.

4.11.6 Conclusions on classification and labelling

Pencycuron does not need to be classified for reproduction toxicity.

RAC evaluation of reproductive toxicity
Summary of the Dossier submitter's proposal One extensively described 2-generation study in rats was available, in which no indication of reproduction toxicity was found. In the 21-day dermal repeated dose toxicity study in rats, an increase in focal distension of the tubules of the testes was observed at the highest dose. However, no clear pattern was observed. In addition, such effects were not observed in other repeated dose studies or in the 2-generation study. Two developmental/teratogenicity studies were available, one performed in rats and the other in rabbits. No treatment-related effects on maternal or litter parameters, including external fetal observations, were found in either of these studies,. No indication of a teratogenic potential of pencycuron in rats or rabbits was observed. In the 2-generation study, some developmental effects were observed at the highest dose level in the form of reduced body weights. However, the maternal body weight was also reduced at this dose

level.
No classification was proposed by the DS for fertility or developmental toxicity.

Comments received during public consultation

Industry provided editorial comments on the description of the rat prenatal development toxicity study.

Additional key elements

Fertility

In a 2-generation study (Tauchi, 1990), Wistar rats were administered 50, 500 or 10000 mg/kg pencycuron in diet (approx. 3.2/4.6, 32/49 and 676/999 mg/kg bw/d in males/females, respectively). The study was performed in accordance with OECD TG 416 except that examination of some organs was not performed in the pups (brain, pituitary, thyroid, adrenal glands, prostate and seminal vesicles). The F0 and F1 generations were both mated twice.

Body weight was significantly decreased in a transient way in F0 females at 49 mg/kg bw/d and in F0 males from 32 mg/kg bw/d and permanently in high dose females as compared to controls. Effects on liver weight (relative increase at mid-dose in F0 females; absolute and relative increase from mid dose in F0 males and F1 females and at high dose in F1 females), kidney (increase in all exposed F0 females groups) and spleen (decrease at high dose in F0 and F1males and females) were observed. Magnitudes were not given. Microscopic findings were restricted to centrilobular swelling of the liver in high dose F0 and F1 animals.
No effects were observed on mating performance, fertility, gestation index or length, litter size, sex ratio or offspring survival.

Effects on the reproductive organs were restricted to a significant increase in the absolute and relative ovarian weight from mid-dose in F0 females and a decrease in absolute testicular weight in high dose F0 males. Effects on pup body weights were noted in high dose F1 pups and in F2 pups from 32/49 mg/kg bw/d. The main findings related to reproductive toxicity are summarised in Table 7 below.
Table 7 – Findings on reproductive organs in the 2-generation study in rats

	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Dose (mg/kg bw/d)	**0**	**0**	**3.2**	**4.6**	**32**	**49**	**676**	**999**
F0 animals								
Body weight					dc[1]	dc[1]	dc[1]	dc
Food consumption					dc[1]		dc[1]	dc[1]
Testis weights (mg)								
Absolute ± SD	3191 ±187		3157 ±176		3151 ±327		**3090 ±172***	
Relative ± SD	560 ±50		550 ±35		549 ±60		548 ±41	
Ovary weights (mg)								
Absolute ± SD		109 ±19		110 ±18		**129 ±35***		**124 ±21***
Relative ± SD		32 ±6		32 ±6		**39 ±10***		**39 ±6***

dc=statistically significantly decreased compared to the controls
[1]=over a distinct, but not the whole, treatment period
***=statistically significant, p<0.05**

Table 8 – Findings on pups body weights in the 2-generation study in rats

Dose (mg/kg bw/d)	**0**	**3.2/4.6**	**32/49**	**676/999**
F1a - PND 0				
Maternal bw ± SD	293±14	301±25	286±24	**262±18* (-11%)**
Pup body weight ± SD (females)	6.2±0.6	6.0±0.7	6.2±0.7	**5.8±0.7* (-7%)**
Pup body weight ± SD (males)	6.5±0.6	6.3±0.7	6.6±0.7	**6.1±0.7* (-6%)**
F1a - PND 21				
Maternal bw ± SD	311±14	318±24	**301±19* (-3%)**	**279±13* (-10%)**
Pup body weight ± SD (females)	45.3±4.6	45.7±5.7	43.1±3.6	**36.9±3.2* (-19%)**
Pup body weight ± SD (males)	47.9±4.9	46.4±6.5	46.0±3.3	**38.6±3.1* (-20%)**
F1b - PND 0				
Maternal bw ± SD	310±19	**327±32***	304±25	**291±17* (-6%)**
Pup body weight ± SD (females)	6.2±0.7	6.1±0.7	6.1±0.8	**5.8±0.9* (-7%)**
Pup body weight ± SD (males)	6.6±0.7	6.6±0.8	6.5±0.9	**6.1±1.0* (-8%)**
F1b - PND 21				
Maternal bw ± SD	345±15	349±21	**329±25* (-5%)**	**320±14* (-7%)**
Pup body weight ± SD (females)	41.9±7.1	43.1±7.2	40.9±5.5	**38.7±4.2* (-8%)**
Pup body weight ± SD (males)	46.4±5.7	44.9±9.4	43.4±4.9	**40.4±5.0* (-13%)**
F2a - PND 0				
Maternal bw ± SD	298±24	303±22	290±25	284±25
Pup body weight ± SD (females)	6.3±0.5	6.1±0.4	6.0±0.7	**5.8±0.6* (-8%)**
Pup body weight ± SD (males)	6.7±0.5	6.6±0.5	6.3±0.7	**6.3±0.7* (-6%)**
F2a - PND 21				
Maternal bw ± SD	320±24	327±22	317±20	311±28
Pup body weight ± SD (females)	50.2±7.1	48.6±3.9	**45.9±4.8* (-9%)**	**37.3±4.0* (-26%)**
Pup body weight ± SD (males)	52.6±7.5	50.6±4.7	48.7±6.5	**40.7±4.5* (-23%)**
F2b - PND 0				
Maternal bw ± SD	325±27	338±29	323±23	328±31
Pup body weight ± SD (females)	6.4±0.5	6.3±0.7	6.5±0.5	6.4±0.7
Pup body weight ± SD (males)	6.7±0.6	6.8±0.7	7.0±0.6	6.9±0.7
F2b - PND 21				
Maternal bw ± SD	344±23	351±30	343±22	335±25
Pup body weight ± SD (females)	50.1±5.6	48.0±5.9	**45.2±6.2* (-10%)**	**38.8±3.9* (-23%)**
Pup body weight ± SD (males)	51.8±4.4	50.9±6.2	50.3±6.2	**42.0±4.2* (-19%)**

***=statistically significant**

Developmental toxicity

In addition to the 2-generation study performed in rats (see above), prenatal toxicity studies were performed in rat and rabbit and they are very briefly described in the CLH report.

In rabbits (Becker, 1983), oral administration of pencycuron at doses of 200, 600 or 2000 mg/kg bw/d from gestation days 6-18 in a study compliant with OECD TG 414 (lacking the macroscopic examination of the dams) resulted in an increased foetal weight in the high dose group. The effect was significant on a litter basis but not on an individual basis and this finding is not considered toxicologically significant. Maternal toxicity was not present in this study.

In rats (Langrand-Lercher, 2008), oral administration (gavage) of pencycuron at 40, 200 or 1000 mg/kg bw/d from gestation days 6-20 in a study compliant with OECD TG 414 did not affect maternal parameters at any of the doses tested. It has no impact on the number of corpora lutea, pre- or post-implantation loss, sex ratio, foetal weight or number of live fetuses.

Increased incidences of some skeletal and visceral findings were noted in the high dose fetuses and are summarized in Table 9 below. Statistical significance was not reported for any of these findings.

Table 9 – Incidence (%) of foetal findings in the rat prenatal toxicity study

Dose (mg/kg bw/d)	**Incidence basis**	**0**	**40**	**200**	**1000**	**Historical control data**
Skeletal findings -incomplete ossification hyoid centrum	Litter	4.8	10.0	13.0	13.0	0-12.5%
	Foetus	1.4	1.4	1.8	2.9	0-1.6%
-unossified 7th cervival centrum	Litter	14.3	0.0	13.0	17.4	0-41.7%
	Foetus	4.8	0.0	2.9	2.3	0-12.2%
-discontinuous costal cartilage	Litter	33.3	35.0	21.7	39.1	9.1-38.1%
	Foetus	4.8	5.5	4.7	5.8	1.3-8.4%
-incomplete ossification 5th metacarpals	Litter	4.8	0.0	4.3	8.7	0-9.1%
	Foetus	1.4	0.0	1.2	1.7	0-3.2%
-less than 9 sacrocaudal vertebrae ossified	Litter	4.8	5.0	0.0	13.0	0-20.0%
	Foetus	2.0	0.7	0.0	2.3	0-3.7%
Visceral findings - renal pelvis dilatation	Litter	0	0	0	8.7	0-8.3%
	Foetus	0	0	0	1.2	0-1.2%
- dilated ureters	Litter	42.9	40.0	47.8	52.2	30.4-75.0%
	Foetus	11.7	11.6	12.9	11.2	7.4-29.7%

A third prenatal developmental toxicity study performed in rats was mentionned in the CLH report but was not further described and considered because of high mortality in control and exposed animals and because of methodological limitations.

Assessment and comparison with the classification criteria

Fertility

In a 2-generation guideline study in rats, no effects on fertility were observed.
The decrease in absolute testicular weight in high-dose males was not significant when the relative weight was considered. It was not accompanied by histopathological or functional findings and it was not observed in the F1 and F2 generations.

In the repeated dose toxicity studies, focal distension in testicular tubules was observed in the 21-day rabbit dermal study in all test groups (0/5, 2/5, 2/5 and 3/5 males exposed to 0, 250, 500 or 1000 mg/kg bw/d with unilateral or bilateral tubular distension). However, incidences did not reveal a clear dose-response and relation to treatment is uncertain. Repeated-dose toxicity performed in the rat, mouse and dog did not reveal effects on the

testis or in any male or female reproductive organs.

In the 2-generation study, a significant decrease in absolute and relative ovarian weight was reported in F0 females exposed to 49 and 999 mg/kg bw/d. It was not accompanied by histopathological or functional findings and it was not observed in the the F1 and F2 generations. Besides, no clear dose-response was observed.

Overall, RAC considers that data do not provide evidence that pencycuron induces adverse effects on the reproductive organs or on fertility and no classification is supported for fertility.

Developmental toxicity

No developmental effects were observed in a rabbit prenatal developmental toxicity study.

Visceral and skeletal findings were observed in the rat prenatal developmental toxicity study in the absence of maternal toxicity. Incidences of unossified 7th cervical centrum, incomplete ossification of the 5th metacarpals, delayed ossification of sacrocaudal vertebrae and dilated ureters were within the historical control data (HCD) ranges both on a litter and on a foetal basis and were not considered related to treatment.

The incidence of discontinuous costal cartilage was slightly above the HCD at the high dose on a litter basis only and no dose-response is observed. Because the high dose incidence was only slightly above the incidence in the controls and low dose incidence was also near the upper limit of historical controls, RAC did not consider this finding as treatment-related.

The incidence of renal pelvis dilatation was slightly but clearly above the HCD at the high dose on a litter basis only. Solecki *et al.* (2003) noted that the incidence of dilated renal pelvis was generally greater in fetuses than in pups supporting the idea that these changes are likely to be transient and should be considered as variation. Solecki *et al.* (2003) also concluded with a good agreement score (75%) that it was a variation and not a malformation. The slight increase in incidence of dilated renal pelvis is therefore not considered by RAC as sufficient to justify classification.

The incidence of incomplete ossification of hyoid centrum was at the upper range of historical controls from 200 mg/kg bw/d both on a litter and on a foetal basis and exceeded the HCD at the high dose on a foetal basis. This finding was unanimously considered as a variation in the report of the third workshop on the terminology in developmental toxicology (Solecki, 2001).

Makris *et al.* (2009[4]) confirmed that this finding relates to the ossification status and does not involve an abnormality of the structure. Although it is noted that this delay in ossification is observed without a significant effect on body weight development, this finding is not considered by RAC as sufficiently severe to justify a classification.

An effect on pup weight was observed in the 2-generation study in rats. Whereas there was either no effect on pup body weight at birth (F2b) or a decrease of similar magnitude than maternal body weight decrease (F1a, F1b, F2a), the effect was more pronounced at the end of the lactation period except for F1b.

In F1a, although the decrease in pup body weight on postnatal day (PND) 21 was of higher magnitude that the decrease in maternal body weight (-10% in dams vs. -19/20% in pups), the decrease in maternal body weight indicated some maternal toxicity and it could

[4] Makris S. *et al*. (2009) Terminology of developmental abnormalities in common laboratory mammals (Version 2). *Reprod Toxicol* **86**(4):227–327

not be excluded that the effect on pup weight may be secondary to maternal toxicity.

In F2a and F2b, no effect on maternal body weight was observed at the end of the lactation period. The decrease in pup body weight was observed from mid-dose in females and increased with dose, it was also observed at high dose in males. It was noted that the weight of control F2 pups at the end of the lactation period was high compared to control F1 pups. Compared to F1b control pups, there was no decrease in F2 female mid-dose pup weight and the decrease in high dose pups was still present but reduced to 7 to 12%. Historical control data were not available to confirm that F2 control pup weight was unusual high at the end of the lactation period.

No data is available on a possible presence of pencycuron in milk or on a possible impairment of milk quality or quantity. Overal during the lactation period, a progressive decrease in pup body weight (no or slightly decreased body weight at birth, an effect that worsened towards the end of the lactation period) was observed. This suggests that the effect was likely a consequence of a direct exposure of pups via food. The effect therefore indicated that pups were more sensitive to body weight impairment than dams in terms of magnitude of the observed effect. However, the results of the studies did not indicate that the effect on pup body weight occurred at lower doses than the doses also inducing body weight impairment in dams although to a lesser extent.

Overall, RAC considers that the effect of pencycuron on the impairment of pup body weight development during lactation at the highest dose does not justify a classification of pencycuron for developmental toxicity. RAC supports no classification for developmental toxicity.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Pencycuron was orally administered to four groups of 12 rats/sex/dose in a 13-weeks diet study according to OECD 424. Dose levels were 0, 500, 2500, and 15000 mg/kg food (equal to 0, 35, 181, and 1168 mg/kg bw/d for males and 0, 51, 275, and 1836 mg/kg bw/d for females) and based on previous performed studies (Schladt and Lawrence, 2006).

Table 41 Results of a neurotoxicity study in rats.

Dose (ppm food)	0		500		2500		15000		dr
	m	f	m	f	m	f	m	f	
Mortality	no mortality								
Clinical signs[1]									
- Spastic gait (week 5-6)	0/12	0/12	2/12	2/12	3/12	3/12	5/12	9/12	m,f
- High-stepping gait (week 6-10)	0/12	0/12	2/12	4/12	3/12	1/12	1/12	6/12	
- Uncoordinated gait (week 9-10)	0/12	0/12	1/12	1/12	2/12	0/12	3/12	0/12	
Body weight gain					dc[2]	d[2]	dc[2]	dc[2]	m,f [2]
Food intake						i[3]	ic	ic	m,f
Water intake						ic		ic	f
Functional observation battery [4]									
- Spastic gait (week 5)	0/12	0/12	1/12	2/12	3/12	3/12	5/12	9/12	m,f
- Stilted hindlimbs (week 8[5])	0/12	0/12	4/12	8/12*	3/12	3/12	2/12	5/12	
- Walking on tiptoe (week 13)	0/12	0/12	1/12	0/12	0/12	0/12	0/12	0/12	
Motor activity									
- Motor activity								i[6]	

Dose (ppm food)	0		500		2500		15000		dr
	m	f	m	f	m	f	m	f	
- Locomotor activity								i[7]	
Ophthalmology	no treatment-related findings								
Brain weights	no treatment-related findings								
Pathology									
macroscopy	no neurotoxicity-related findings [8]								
microscopy	no treatment-related findings								

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
1 Hair loss, a thin haircoat, an injury and/or a wound were also observed among animals.
2 During week 1 only, for the remaining study duration, body weight gain was similar for controls and dose groups.
3 Achieving statistical significance over Days 50-57 only.
4 No treatment-related findings were observed for other functional observations such as grip strength, foot splay and reflex testing.
5 Also observed in week 4 for one male and one female at 500 ppm and in week 13 for one male at 2500 ppm and one female at 15000 ppm.
6 Achieving statistical significance in week 2.
7 Achieving statistical significance in week 8.
8 5/12 females at 15000 ppm developed distinct liver lobulation, which was not examined histopathologically.
***** statistically significant (p≤0.05)

The body weight gain of males and females at 2500 and 15000 mg/kg food during week one is considered to be not adverse because body weight gain of all male and female rats over the entire study period was similar to control. Several gait abnormalities were observed during the study, including spastic gait, high-stepping gate, uncoordinated gait, stilted hindlimbs and walking on tiptoe. These effects were not observed in control animals, however, these effects were transient and occurred in the absence of concomitant findings such as hindlimb foot splay and histopathology findings. Furthermore, no clear dose-dependent relationship was observed for these effects, except for spastic gait in females in week 5. Therefore, the gait abnormalities are considered not to be of a toxicologically relevant adverse nature.

Both motor and locomotor activity appeared increased for both males and females of all dose groups during the study period, but were not dose-related and were within the range of historical reference except for the activities of half of the females at 15000 ppm. Although achieving statistical significance at week 2 and 8, the increased activities in females at 15000 ppm did not progress in incidence or severity with time.

In this subchronic dietary neurotoxicity study, several treatment-related effects were observed which are considered not to be of a neurotoxicologically relevant adverse nature. Based on the increased motor and locomotor activities in females at 15000 mg/kg food, the NOAEL is set at 2500 mg/kg food (equal to 181 mg/kg bw/day in males and 275 mg/kg bw/day in females).

4.12.1.2 Immunotoxicity

No information is available.

4.12.1.3 Specific investigations: other studies

A short summary of an in-vitro study investigating the influence of pencycuron on histamine release from mastocytes is mentioned in the DAR. However, this summary is only one page and was not evaluated and summarised in the DAR.

4.12.1.4 Human information

No information available.

4.12.2 Summary and discussion

In a 13-week neurotoxicity study in rats, increased motor and locomotor activities were observed in females at 1836 mg/kg bw/day. No neurotoxicity was observed at 181 and 275 mg/kg bw/day in respectively, males and females.

4.12.3 Comparison with criteria

The results of the neurotoxicity study are compared with the criteria for STOT RE. These criteria require significant toxicity at dose levels of 100 mg/kg bw/day or below in a 90-day study. However, as the NOAEL was above 100 mg/kg bw/day, the criteria are not fulfilled.

4.12.4 Conclusions on classification and labelling

No classification for STOT RE is required based on the available 90-day neurotoxicity study in rats.

RAC evaluation of aspiration toxicity
Summary of the Dossier submitter's proposal The DS did not address classification for aspiration toxicity in the CLH dossier. **Comments received during public consultation** No specific comments were received. **Assessment and comparison with the classification criteria** Pencycuron is a solid and classification for aspiration toxicity is not relevant for solid substances according to section 3.10.1.6.2 *bis* of the CLP regulation. RAC considers that no classification for aspiration toxicity is justified.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental hazards of pencycuron were assessed in the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of pencycuron in Annex I of Council Directive 1107/2009/EEC (Draft Assessment Report, November 2009 and updated May 2010 concerning the placing of plant protection product on the market.

The bioaccumulation study of Oyama, Araki and Takase (1982) was reviewed for the purpose of this proposal. This is the only study report that was reviewed. Other summaries included in this proposal are copied from the DAR (and its addenda and assessment reports when these contain updated information). For an overview of the hazard property being evaluated, all reliable information relating to that property has been summarized in a table. Detailed information is only included for the key study used to derive the classification. References to individual studies are not included. For more details, the reader is referred to the DAR and its addenda.

5.1 Degradation

Table 42 Summary of relevant information on degradation

Method	Results	Remarks	Reference
[Methylene-^{14}C]pencycuron hydrolysis at 25°C , EPA guideline	DT50 (pH 5) > 30 days DT50 (pH 7 and 9) stable	Hydrolytically stable	DAR, (Daly and Heim, 1999)
[Methylene-^{14}C]pencycuron hydrolysis at 50°C , EC. C7 guideline	DT50 (pH 5) = 4.7 days DT50 (pH 7) = 14.5 days DT50 (pH 9) = 15.4 days		DAR (Hellpointer, 2002)
Hydrolysis of pencycuron and pencycuron-PB-amine. Recalculated from study of Hellpointer (at 50°C , EC. C7 guideline).	Pencycuron DT50 (pH5) 4.7 days DT50 (pH 7) 14.4 days DT50 (pH 9) 15.0 days Pencycuron-PB-amine pH5 and pH 7: stable DT50 (pH9) 70 days	Using software programme	DAR (Görlitz and Mikolasch, 2003)
14C labeled pencycuron [M-label and pH-label] at 50 °C, 25 °C and 20 °C. OECD 111 guideline	DT50 (pH 4) and 50 °C: 20.5 hours. DT50 (pH 4) and 25 °C: 20.2 days DT50 (pH 4) and 20 °C: 29.9 days DT50 (pH 7 – 9) and 50 °C: 8.2 – 9.1 days. DT50 (pH 7 – 9) and 20 – 25 °C: 194 – 289 days.		DAR (Heinemaan, 2008)
Pencycuron, photodegradation, UBA guideline.	Photodegradation is not expected	Absorption coefficient < 10 L/mole*cm at 291 nm	DAR (Hellpointer, 1990)
Pencycuron, water/sediment simulation study	DT50 system: 82.6 and 139.0 days (geometric mean: 107.2 days) at 22 °C.	Two systems, no degradation producs > 10% AR.	DAR (Scholz, K. and Freymiller, U. 1986)

5.1.1 Stability

Hydrolysis

A hydrolysis study with radiolabelled pencycuron (chemical purity 98.9%, radiochemical purity 97.3%) was conducted according to EPA guideline and GLP status. Methylene-^{14}C pencycuron was incubated in sterile buffer solutions at three pH levels (pH 5, 7, and 9) at 25°C and at one concentration (0.20 µg/mL). The test at pH 7 was performed with two buffer solutions: TRIS buffer and a HEPES buffer. Recoveries of the applied radioactivity were > 94%. At pH 5, pencycuron was relatively stable until day 21 but then degraded for 40% by day 30. Pencycuron is hydrolytically stable at pH 7 and 9 at 25°C. DT50 values were 64, 131, 158, and 302 days at pH 5, pH7 (TRIS buffer), pH 7 (HEPES buffer), and pH 9, respectively. Calculated DT50 value at pH5 is insufficiently reliable in

view of the irregular pattern of degradation. Conclusion: DT50 at pH 5 > 30 days. Degradation products were not identified as no analytical standards were available.

A second hydrolysis study with radiolabelled pencycuron (chemical purity > 98%, radiochemical purity ≥ 99%) was conducted according to EC C.7 and SETAC guideline and GLP status (Hellpointer,2002). Methylene-^{14}C pencycuron was incubated in sterile buffer solutions at three pH levels (PH 5, 7, and 9) at 50°C and at one concentration (0.2 mg/L) for 10 days. Recoveries of the applied radioactivity varied between 97.8 and 102%.The only degradation product found was pencycuron-PB-amine, for which a maximum amount at pH5 of about 86% of the applied radioactivity after 10 days was found at 50 °C. DT50 values,recalculated by the RMS according to the EU regulation (SANCO/10058/2005, version 1.0), were 4.7, 14.5, and 15.4 days at pH 5, pH7, and pH 9, respectively.

The DT50 values of pencycuron and its degradation product pencycuron-PB-amine of the last study were recalculated by Görlitz and Mikolasch, (2003) using the ACSL Optimize software (1996) and according to EPA guideline. The hydrolytic degradation of pencycuron followed 1st order kinetics. The recalculated values were as follows: for pencycuron at pH5 4.7 days, pH7 14.4 days, and pH9 15.0 days. The degradation product pencycuron-PB-amine was stable at pH5 and pH7 and a DT50 value of 70 days was calculated at pH9.

Heinemann (2008) performed a hydrolysis study with methylene-^{14}C-pencycuron (chemical and radiochemical purity > 98%, M-label and Ph-label) at 50, 25 and 20°C in buffered solutions at pH 4, 7, and 9 according to OECD 111 guideline. Test concentration was 0.15 mg/L. Test solutions were incubated in the dark. Duplicate samples were taken at 0 h and at different time points up to 48 hours (pH 4, 50 °C), up to 21 days (pH 7 and 9, 50 °C) and up to 30 days (pH 4, 7, and 9, 25 °C and 20 °C) . Analysis was by LSC and direct HPLC-MS and HPLC-MS/MS for identification of the parent compound and the degradation product pencycuron-PB-amine. The recoveries ranged from 89 – 112%. Maximum levels of pencycuron-PB-amine were 73 – 96% RA (radioactivity) at 50 °C (pH 4, 7, and 9), 53-71% RA at 20 °C (pH 4) and 25 °C (pH 4), 9 – 11% AR at 25 °C (pH 7 and 9), and 6 – 7% AR at 20 °C (pH 7 and 9). Maximum levels of aniline were 42 – 80% AR at 50 °C (pH 4, 7, and 9), 49 – 64% AR at 20 °C (pH 4) and 25 °C (pH 4), 6 – 9% AR at 25 °C (pH 7 and 9), and 3 – 4% AR at 20 °C (pH 7 and 9). These results showed that pencycuron was hydrolytically instable at pH 4 (DT50 of 20.5 hours, 20.2 days and 29.9 days at 50 °C, 25 °C and 20 °C, respectively). At pH 7 – 9, DT50 values were 8.2 – 9.1 days at 50 °C, 194 – 289 days at 20 – 25 °C. DT50 values of the degradation products were not calculated.

Photodegradation in water.

In a photodegradation study (Hellpointer, 1990), the absorption of light at > 295 nm was measured to give an indication whether photo-degradation of pencycuron (purity 99.3%) may be expected. UV-absorption properties were characterised in a solution of 11.5 mg pencycuron/L in water/acetonitrile 80 : 20. The absorption coefficient was < 10 L/mole*cm at 291 nm, which indicates that photodegradation is not expected. Therefore, pencycuron is not considered to be directly photodegradable.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

A ready biodegradability test in not available.

5.1.2.3 Simulation tests

Water/sediment.

One reliable aerobic water/sediment study with two types of aerobic water-sediment systems is available (Scholz and Freymiller, 1986). One system was collected from a ditch of an agriculture facility (IJzendoorn) and the other system was collected from a fish pond (Lienden, NL). Sediment samples were wet sieved (2 mm) and dry weight was determined, water was filtered. Vessels were filled with 1.3 cm sediment and 400 mL water. Systems were equilibrated for 14 days. A mixture of radiolabelled [cyclopentyl-3,5-^{14}C]pencycuron (radiochemical purity 98%) and unlabelled pencycuron (purity 99.8%) was prepared in methanol and 100 µL was added onto the water layer of each system to give a final application of 0.4 mg/L. 8 vessels were used for each system. The systems were incubated at 22 °C in the dark. Water and sediment were sampled in two vessels after 14, 30, 63 and 91 days. Volatiles were trapped in soda lime. Sediment and water were separated by decantation. Water was analysed by TLC after extraction with ethyl acetate. Sediment was extracted with ethanol and ethyl acetate and analysed by TLC. Bound residues were determined by LSC after combustion. The amount of radioactivity was determined before and after each processing step by LSC. Degradation products were identified.

A day 0 measurement is lacking. In the IJzendoorn system, the total recovery of the radioactivity (RA) varied between 102 – 110%. After 91 days, 91.1% of the applied RA was recovered in the sediment (61.7% extractable and 29.5% un-extractable). In the Lienden system, total recovery was 96.7% - 106% of the RA. After 91 days, 60% of the applied RA was recovered in the sediment (36.2% extractable and 23.9% un-extractable). Mineralisation reached maximum levels of 12% and 22% AR in the IJzendoorn and Lienden systems, respectively, after 91 days. The levels of pencycuron in the total water/sediment system decreased to 39% for Lienden and 55% for IJzendoorn after 91 days. The levels of parent pencycuron reached a maximum in sediment of 55% (Lienden) – 79% (Ijzendoorn) AR on day 30 and decreased to 33% (Lienden) – 54% (IJzendoorn) AR after 91 days. In water, the levels of parent pencycuron were 25% (Lienden) – 3.9% (Ijzendoorn) AR on day 14 and 5.6% (Lienden)- 1.2% (IJzendoorn) AR after 91 days. The mass balance on all study days (day 14 – 91) were adequate (97 – 110 Three minor degradation products were found in the water and sediment phase, which were all < 10% of applied radioactivity (AR). The degradation product pencycuron-PB-amine was found at maximum levels of 3.5% of AR in water and 7.2% of AR in sediment. DT50 values were modelled and calculated according to the guidance in FOCUS (2005,SANCO/1058/2005, version 1.0), (Ref. Hammel and Kahl, 2009). The following DT_{50} values were calculated at 22 ºC

Level [1]	compartment	system	DT50 at 22°C (days)	Process
I	Whole system	IJzendoorn	139.0	Degradation
		Lienden	82.6	Degradation
	Water	IJzendoorn	0.11	Degradation & mass-transfer
		Lienden	3.8	Degradation & mass-transfer
	sediment	IJzendoorn	152.5	Degradation & mass-transfer
		Lienden	87.0	Degradation & mass-transfer
II	Water	IJzendoorn	30.7	Degradation
		Lienden	25.8	Degradation
	sediment	IJzendoorn	139.7	Degradation
		Lienden	182.5	Degradation

1: level I: modelled the degradation from the whole system, water column and sediment. One-compartment approach.

Level II: degradation rates in water column and sediment are modelled, two-compartment approach..

Dissipation of pencycuron from the water phase was influenced by sorption.

The DT50 calculated for the whole system was 82.6 days in the Lienden system and 139.0 days in the IJzendoorn system.

5.1.3 Summary and discussion of degradation

Several studies on hydrolysis indicate that at 20 °C pencycuron is stable at pH 7 and 9, but is susceptible for hydrolysis at lower pH, with a DT50 value of around 30 days at pH 4.

Pencycuron is not considered to be directly photodegradable in one photodegradation study.

No ready biodegradability study is available.

In an aerobic water-sediment study using two systems, the calculated DT50 for the whole system was 82.6 - 139.0 days at 22 °C (equivalent to 99.1 - 166.7 days at 20 °C). I In these aerobic water-sediment systems, mineralisation reached maximum levels of 12%- 22% after 91 days. Based on limited hydrolysis, lack of photolysis and a DT50 (whole system) of >16 days in aerobic water-sediment studies it can be concluded that pencycuron does not undergo rapid primary degradation in the environment. Furthermore, pencycuron underwent limited mineralisation in two water-sediment systems (maximal 22% mineralisation after 91 days). In conclusion, pencycuron is considered to be not rapidly degradable in the aquatic environment according to the CLP regulation as it failed to meet the criterion of >70% degradation in 28 days.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

The sorption of radiolabelled (methylene-^{14}C) pencycuron (radiochemical purity >99.9%) was determined in a batch equilibrium study with four soils (Daly, 1989). Soil samples (1 g dw of soil) were shaken with test solution (0.0179, 0.0915, 0.143 and 0.186 mg/L, measured). After six hours of

shaking, the tubes were centrifuged and supernatant was removed. The radioactivity of the supernatant was determined by LSC analysis and the stability of the test compound by TLC. The total radioactivity recoveries ranged from 91.7 to 106%. A summary of the results is given in the table below.

Table43 Adsorption coefficients of Pencycuron in four soils

Soil type	% organic carbon	adsorption in L/kg		
		Kd	Koc	1/n
sand	0.526	55.0	10441	1.1770
sandy loam	0.577	28.4	4912	1.0519
silt loam	1.53	36.9	2414	0.9058
clay loam	1.16	56.7	4899	1.2058

The TLC analysis at test initiation and after equilibrium showed that pencycuron was not stable in the test solution. The amount of test material adsorbed to soil was calculated as the difference between the amount of radioactivity present in the adsorption solution after adsorption and that initially added to each tube. Since this method involves no correction for degradation in the aqueous phase, the amount of pencycuron adsorbed to soil was underestimated and the equilibrium concentration of pencycuron overestimated by this method. Hence the adsorption coefficients are underestimated. Although, there is some doubt about the accuracy of the reported Freundlich adsorption coefficients, they are accepted as worst case values.

The sorption of radiolabelled [methylene-^{14}C] pencycuron-PB-amine (a metabolite of pencycuron) was determined in a batch equilibrium study with four soils according to OECD 106 guideline (Fent, 1998). Soils were air dried and sieved (2 mm). Soil samples (range: 1 - 9 g dw of soil) were shaken with 20 mL test solution (nominal concentrations 0.04, 0.20, 1.06 and 5.39 mg/L). After 24 hours of shaking, tubes were centrifuged and supernatant was removed. The radioactivity of the supernatant was determined by LSC analysis and the stability of the test compound by HPLC. The total radioactivity recoveries ranged from 92 to 107%. The HPLC of the supernatants showed that > 87% of the radioactivity in the supernatants represented unchanged test substance. A summary of the results is given in the table below.

Table 44: Adsorption coefficients of [methylene-^{14}C]pencycuron-PB-amine in four soils

Soil type	% organic carbon	adsorption in L/kg		
		Kd	Koc	1/n
sandy loam	1.80	8.12	451.3	0.80
loamy sand	2.48	5.12	206.6	0.85
sand	0.70	1.11	158.3	0.84
loam	1.98	40.72	2056.6	0.91

5.2.2 Volatilisation

The most reliable vapour pressure for pencycuron is 5.10^{-10} Pa at 20° C (extrapolated value) and the calculated Henry's law constant at these vapour pressure is 5.10^{-7} Pa.m^3.mol^{-1} at 20 °C. Based on this information it is concluded that significant volatilisation of pencycuron does not occur.

5.2.3 Distribution modelling

No information available.

5.3 Aquatic Bioaccumulation

Table 45: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
BCF study with carp, no guideline (not available at the moment of performance)	BCF = 226 L/kg, not lipid corrected	Study is considered not reliable.	DAR (Oyama, Araki and Takase, 1982)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation.

The log Kow of pencycuron is 4.0 at 25 °C and 4.7 at 20 °C.

5.3.1.2 Measured bioaccumulation data

In a bioaccumulation test of pencycuron (purity 99%) in carp, the fish (a total of 20) were exposed for 28 days with a 14 days depuration period (Oyama, Araki and Takase, 1982). A continuous flow-through exposure regime was carried out at 0.1 mg/L nominal. No blank control was included. Samples from fish were taken on exposure days 3, 7, 14, 21 and 28 and during depuration after 3, 7, and 14 days. Water was collected daily. Test substance concentrations were analysed by HPLC in the water and by GLC in the fish tissue. Analytical recoveries of pencycuron from water and fish were 98% and 80%, respectively. Measured concentration in the water was 0.084 mg/L. Pencycuron was detected in the fish tissue. Approximately 90% of the pencycuron was excreted within 3 days; after 7 days of depuration, pencycuron was no longer detected in the fish (detection limit 0.01 mg/L). The highest BCF was 226 L/kg, not corrected for lipid content. The lipid content in fish was 4%. The BCF was calculated as the ratio of the concentration of pencycuron in fish versus water.

This study has deficiencies: lethal and sublethal effects were not recorded, growth of fish during the study was not taken into account; there is a great difference between the concentration of pencycuron found in the two fish for the same time points and identification of metabolites was not performed. The GC-chromatogram of the standard stock solution shows several degradation products (all hydrophilic). In the chromatogram of the fish, only pencycuron and one (minor) metabolite is visible. It is not clearly described in the report which concentration for pencycuron in water was taken into account for the calculation of the BCF. Therefore, although the study was well conducted at the time it was performed (1982), the resulting BCF is questionable and the study quality is such that it cannot be used for classification and labelling purposes.

5.3.2 Summary and discussion of aquatic bioaccumulation

Pencycuron has a log Kow of 4.0 – 4.7 at 20 – 25 °C. One bioaccumulation study in carp is available. However, due to significant methodological deficiencies in this study, the BCF value for pencycuron derived in this study is considered not reliable and cannot be used for classification purposes. Pencycuron is considered to fulfil the criterion for bioaccumulation potential according to Regulation EC1272/2008, 2nd ATP, since the log Kow is value is $\geq$ 4.

As metabolites were not identified no judgement can be made on their bioaccumulation potential.

5.4 Aquatic toxicity

Table 46: Summary of relevant information on aquatic toxicity for pencycuron.

Method	Results	Remarks	Reference
Acute fish pencycuron, EPA, OECD guideline	LC_{50} > 0.3 mg a.s./L (water solubility limit).	96-h semi-static, limit test. *Oncorhynchus mykiss*	DAR (Dorgerloh, Sommer, 2001)
Acute fish pencycuron, EPA, OECD guideline	LC_{50} > 0.3 mg a.s./L (water solubility limit).	96-h semi-static, limit test. *Lepomis macrochirus*	DAR (Dorgerloh, Sommer, 2001)
Acute fish pencycuron, EPA guideline	LC_{50} > 0.3 mg a.s./L (water solubility limit).	96-h static test, limit test *Oncorhynchus mykiss*	DAR (Grau, 1990)
Acute fish, pencycuron	LC_{50} > 0.3 mg a.s./L (water solubility limit).	96-h static test *Oncorhynchus mykiss*	DAR (Carlisle, Roney, 1983)
Chronic fish, pencycuron 21 days, OECD 204	21-d NOEC ≥ 0.3 mg a.s./L	21-d semi-static test *Oncorhynchus mykiss*	DAR (Grau, 1989)
Chronic fish, early life cycle: 94 days, OECD 210, pencycuron.	94-d NOEC = 83.2 µg a.s/L. (0.0832 mg a.s./L)	NOEC based on swim-up and growth effects and mean measured concentrations.	DAR (Dorgerloh and Sommer, 2002)
Acute invertebrate, 48-h, OECD 202	48-h LC_{50} > 0.3 mg a.s./L (water solubility limit).	48- h static test *Daphnia magna*	DAR (Hendel, 2001)
Chronic invertebrate, 21-d, pencycuron, OECD 202	21-d overall NOEC: 67.0 µg/ a.s./L (0.067 mg a.s./L).	NOEC is based on parental immobility and mean measured concentrations.	DAR (Memmert, 1991)
Chronic invertebrate, 21-d, pencycuron, OECD 211	21-d overall NOEC: 99.2 µg/ a.s./L (0.0992 mg a.s./L).	NOEC based on reduction in parental body length and nominal concentrations.	DAR (Bruns, 2009)
Algae inhibition, pencycuron, 72-h, OECD 201	E_rC_{50} > 0.3 mg a.s/L (water solubility) NOE_rC ≥ 0.3 mg a.s./L.	72-h static, nominal *Scenedesmus subspicatus*	DAR, (Dorgerloh and Sommer, 2001)

Table 47: Summary of relevant information on aquatic toxicity for pencycuron-PB-amine

Method	Results	Remarks	Reference
Acute fish, pencycuron-PB-amine; OECD 203 guideline	LC_{50} = 29.8 mg a.s./L	96-h static test *Oncorhynchus mykiss*	DAR (Dorgerloh, Sommer, 2002)
Acute invertebrate, pencycuron-PB-amine, 48-h, OECD 202	48-h LC_{50} = 17 mg a.s/L	48-h static test *Daphnia magna*	DAR (Hendel and Sommer, 2001)
Algae inhibition, pencycuron-PB-amine, OECD 201	E_bC_{50} and E_rC_{50} > 8.92 µg/L NOE_bC = 1.75 µg/L; NOE_rC > 8.92 µg/L.	72-h static, measured concentration *Scenedesmus subspicatus*	DAR (Seyfried, 2002)

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

The acute toxicity of pencycuron to fish was tested in different species: *Oncorhynchus mykiss* (three different studies) and in *Lepomis macrochirus*.

In the study with rainbow trout (*Oncorhynchus mykiss*) and with bluegill (*Lepomis macrochirus*) pencycuron (purity 99.3%) was tested under semi-static conditions with a daily replacement. The fish were commercially obtained. The tests were carried out as limit test at the water solubility of pencycuron of 0.3 mg a.s./L, test substance was dissolved in acetone. Chemical analysis was by HPLC.(Dorgerloh, 2001).

Rainbow trout study: Mean measured concentrations of the test substance in fresh and old medium at t=0 and t=1 day was 89% of nominal. The concentrations of the fresh medium at day 1, 2 ,and 3 ranged 100 – 107% of nominal. pH and oxygen values were within acceptable limits. No acute mortality was observed. In the dose group (0.3 mg a.s./L) all surviving fish showed inactivity or laid inactive on the bottom of the vessel. The 96-h LC_{50} is estimated to be > 0.3 mg a.s./L, the water solubility limit.(Dorgerloh, 2001).

Bluegill fish.: The mean measured test concentrations were 87% of nominal. pH and oxygen values were within acceptable limits. No acute mortality was observed. In the dose group (0.3 mg a.s./L) all surviving fish showed inactivity or laid inactive on the bottom of the vessel. The 96-h LC_{50} is estimated to be > 0.3 mg a.s./L, the water solubility limit.(Dorgerloh, 2001).

A second acute toxicity study (Grau, 1990) with pencycuron (purity 93.6%) on *Oncorhynchus mykiss* was performed as a limit test at a nominal concentration of 93.6 mg a.s/L (which is far above the water the solubility) under static conditions according to EPA guideline. One replicate for the control and test concentration with 30 fish each. Test concentration was analysed by HPLC at day 0, 1, 2 and at the end of the study. Un-dissolved material was found. Initial concentration was above the water solubility of the test substance, during the study it decreased. At day 2 and day 4 the measured concentration was 0.26 mg pencycuron/L. Water quality was within acceptable limits. No acute mortality was observed in control or dose group. Sublethal effects were observed in the dose group: all fish showed a slight irregular swimming behaviour, apathy, or were swimming mainly at the bottom of the vessel. It must be assumed that the amount of test substance dosed above the aqueous solubility of the active ingredient is not bioavailable and does not contribute to the exposure of the fish. It is concluded that the 96-h LC_{50} is > 0.3 mg a.s./L (aqueous solubility limit).

In an another acute toxicity study, Carlisle and Roney (1983), exposed the rainbow trout (*Oncorhynchus mykiss*) at nominal concentrations of 0, 150, 220, 320, 470, and 690 mg pencycuron (purity 98%)/L for 96 hours under static conditions. Acetone was used as solvent. One replicate per treatment with 10 fish each. Test concentrations were not analysed during the study. Water quality criteria were within acceptable limits. Cloudiness in the water column and a precipitate on the bottom and d film on the water surface were observed. No acute mortality or any other adverse effects were observed in the control and in the test groups were determined. It was not clear to which extent the nominal concentrations corresponds with the bio-available fraction of the test substance, as the test concentrations were above the solubility of the active ingredient. Un-dissolved particles were visible and actual concentrations were not measured. However, taking the results into account, it must be concluded that the test substance is not toxic to the rainbow trout at a level of its water solubility and this is in line with other fish toxicity studies. 96-h LC_{50} can be set at > 0.3 mg a.s./L, the water solubility level.

Another static 96-h acute toxicity with bluegill sunfish was performed at ten nominal concentrations (10, 32, 47, 69, 100, 150, 220, 320, 470, and 690 mg pencycuron (purity 98%) a.s./L), a water and solvent (acetone) control. One replicate, except 100 mg/L (four replicates) and control, solvent control and 150 mg/L (two replicates). 10 fish per replicate, except at 100 mg/L (15 fish). No analysis of test concentrations. This study is found to be not reliable, because there was no clear dose-response relationship between mortality and test concentration and the dissolved oxygen concentration was far below 60%. In addition, in all dose groups fish showed signs of intoxication with a nonlinear dose-response relationship. Test concentrations were above the water solubility.

Acute toxicity of the degradation product pencycuron-PB-amine to fish

An 96-h acute toxicity study was carried out with pencycuron-PB-amine (purity 98.2%) on the rainbow trout (*Oncorhynchus mykiss*) at five test concentrations (6.14, 12.3, 24.6, 49.1, and 98.2 mg a.s/L, a control and a solvent control (0.5 ml DMF/L) according to OECD 203 under static conditions (Dorgerloh and Sommer, 2002). One replicate per treatment with 10 fish each. Test concentrations were analysed by HPLC at day 0, 2, and 4. The mean measured test concentrations varied between 95% and 98% of nominal (5.91, 11.7, 24.0, 48.0, and 68.7 mg a.s./L). No mortality or any sublethal effect in the control group. In the solvent group one fish died. Mortality was observed at measured concentrations of 24.0, 48.0 and 68.7 mg/L, which was dose-related. Signs of intoxication (laying inactive on the bottom of the vessel, laboured respiration, turning dark in coloration, having convulsions) was observed at 11.7 mg/L and above. The 96-h LC50 is 29.8 mg test substance/L.

5.4.1.2 Long-term toxicity to fish

A 21-day fish prolonged exposure toxicity study (Grau, 1989) was undertaken with rainbow trout (*Oncorhynchus mykiss*) under semi-static conditions with weekly replacement. Juvenile fish (5.8 cm, 2.1 g), one replicate with 10 fish were exposed to pencycuron (purity 98.6%) at one concentration of 98.1 mg/L and a control. Mortality and adverse effects were assessed at least three times a week. Test substance was analysed at day 0, before replacement (day 7) and at the end of the test (day 21). Method of analysis is not reported. The test substance was dosed above its water solubility (0.3 mg/L at 20 °), un-dissolved particles were floating on the surface of the water and settled at the bottom of the vessel. The mean measured test concentrations varied between 18 and 156% of nominal. This variation is related to the dosing above its water solubility. No mortality of any other adverse effect was observed in the control or the only exposure concentration.

Only the results of the chemical analysis are reported and the analytical method is not described. It is not clear to which extent the analytical concentrations correspond with the bio-available fraction of the test substance since the recovery of the active substance was low and variable. However, the test concentration was far above the aqueous solubility of the active ingredient (0.3 mg/L). As no adverse effects to the fish were observed it can be concluded that under the conditions of this test, the test substance is not toxic within its aqueous solubility. It is therefore concluded that the 21-d NOEC with respect to mortality and growth is ≥ 0.3 mg a.s./L (water solubility level).

A 94-day (60-d post hatch) fish early-life stage flow-through study (Dorgerloh and Sommer, 2002) was carried out with rainbow trout (*Oncorhynchus mykiss*). Newly fertilised eggs (1 – 2 hours after fertilisation), four replicates/concentration, 35 eggs/concentration until day 43, from then 15 hatched fish/concentration were exposed to pencycuron (purity 99.4%) according to OECD 210 guideline at nominal concentrations of 9.40, 18.8, 37.5, 75.0, 150, and 300 µg a.s/L, a control and a solvent control (acetone, 0.1 mL/L). Total duration was 94 days (60 days post hatch). Test concentration change regime was 24 changes/day (3.75 L/h). Test concentrations were analysed prior to test, at the start,

weekly during the test and at the end, analysis was by HPLC. Stock solution was also analysed. Mean measured concentrations varied between 107 and 115% of nominal, and were 10.2, 21.7, 40.0, 83.2, 167.4 and 345.1 µg as/L. Water quality was within acceptable values. The mean egg fertilisation in the test concentrations was 99.3%. Percent hatch was not significantly affected in any of the test concentrations compared to control. Percent swim up on day 50 to day 53 was significantly reduced at nominal 150 µg a.s/L (167.4 µg a.s/L measured concentration) and above compared to control, thereafter, significant reduction was only found at the highest concentration up to day 67. The fry growth (length and dry weight) were reduced at nominal 9.40 µg a.s/L (10.2 µg a.s/L measured), in the absence of a dose-response relationship which suggest that the finding at 9.40 µg a.s/L was not related to the test substance. The growth (length and dry weight) was significantly reduced at nominal 150 µg a.s/L (167.4 µg a.s/L measured) and above. The overall 94-d NOEC for pencycuron is set at 83.2 µg a.s/L, based on measured concentrations and swim up and growth effects.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

The acute toxicity to *Daphnia magna* of pencycuron (purity 99.0%) was tested according to OECD 202 guideline under static conditions and 48-h (Hendel, 2001). Ten daphnids (< 24 h old) were used per vessel in triplicate. Eight nominal test concentrations ranged from 0.18 to 10.0 mg a.s./L, a control and a solvent control (0.1 mL acetone/L) were used. Test concentrations were measured at the start and at the end of the study by HPLC. The mean measured test concentrations varied between 17% and 99% of nominal, percentages decreased with increasing nominal test concentrations. Recovery of the active ingredient was < 60% of nominal for test concentrations above 1.8 mg a.s./L at day 2. This is related to the water solubility of pencycuron of 0.3 mg a.s./L. At concentrations above 1.0 mg/L precipitation was observed. In the control and solvent control, immobilised daphnids were < 10%. At 3.2 mg/L and above, adverse effects with respect to condition were observed after 24 and 48 h of exposure. It is assumed that the amount of test substance dosed above the water solubility of the active ingredient is not bio-available and does not contribute to the exposure of the daphnids to the test substance. It can be concluded the test substance is not toxic to *Daphnia magna* within its water solubility (0.3 mg a.s./L). 48-h LC_{50} > 0.3 mg a.s./L.

Acute toxicity of the degradation product pencycuron-PB-amine to invertebrates.

The acute toxicity to *Daphnia magna* of pencycuron-PB-amine (a metabolite of pencycuron, purity 99.0%) was tested according to OECD 202 draft guideline 2000 under static conditions and 48-h (Hendel and Sommer, 2001). Ten daphnids (< 24 h old) were used per vessel in triplicate. Nine nominal test concentrations ranged from 1.0 to 100 mg a.s./L and a control were used. Test concentrations were measured at the start and at the end of the study by HPLC. The mean measured test concentrations varied between 95% and 100% of nominal. No immobility of other adverse effects were observed in the control group. Adverse effects with respect to condition were observed: increase of frequency of antennae movement and daphnids laying at the bottom of the vessel at 5.6 mg test substance and above.. 48-h LC_{50} is set at 17 mg test substance/L.

5.4.2.2. Long-term toxicity to aquatic invertebrates

The chronic toxicity of pencycuron (purity 99.2%) to *Daphnia magna* was determined in a semi-static system for 21-days (Memmert, 1991) according to OECD 202. Daphnids were < 24-h of age at the start. Mortality, reproduction and body length was measured. Control, solvent control (0.1 mg acetone/L) and the test substance concentrations12.4, 24.8, 49.6, 99.2, and 198.4 µg a.s./L were used in the study. The study started with 5 daphnids/ replicate in each vessel, four replicates until day 5,

then the daphnids were individually exposed. Daphnids were fed with algae. Test media were renewed at day 3, 5, 7, 10, 12, 14, 17, and 19 of the study period. Freshly prepared test concentrations were measured on day 0, 12, and 19. Stability of pencycuron was determined under test conditions of freshly prepared test media (without daphnia and algae) of 24.8 and 99.2 µg as/L sampled after 72 hours of incubation. Analysis by HPLC-UV detection at 242 nm after acidification and extraction (method recovery 114.4%). Mortality of the parent, number of offspring and body length of the surviving parents at the end of the study period were determined. NOEC was calculated using Dunnett's test.

The mean measured concentrations in the freshly prepared media were 15.6 (125.8% of nominal), 33.7 (135.9%), 67.0 (135.1%), 125.8 (126.8%), and 183.1 µg as/L (92.3%) for 12.4, 24.8, 49.6, 99.2, and 198.4 µg as/L nominal, respectively. Samples taken at day 12 were 152 – 240% of nominal for the concentrations 12.4 and 99.2 µg as/L and was 83% of nominal for the highest test concentrations. Stock solution range was 81.5 – 109.0% of nominal and the stability samples ranged from 119.2 to 167.2% of nominal. In the control and solvent control mortality was below 20%. Percent survival on day 21 was significantly lower at nominal 99.2 µg a.s./L (65%) and 198.4 µg a.s./L (45%) compared to control. The reproduction rate and the body length of the surviving parents were not significantly different from the control animals. The 21-d NOEC value is set at ≥ 67.0 µg a.s./L mean measured concentration (49.6 µg as/L nominal), and based on immobility of the parent daphnids.

Remark: Water quality parameters were within acceptable levels. Test concentrations were not measured just before renewal (aged solutions), only separate prepared stability solutions (without daphnids and algae). Fresh solutions on day 12 were > 120% of nominal except for the highest concentration. In addition, the mean measured test concentrations were around 125 to 135% of nominal, except the highest concentration. NOEC value should be based on mean measured concentrations. Reliability of the study: 2.

Chronic toxicity study of pencycuron (purity 98.3%) to *Daphnia magna* (≤ 24 hours old) was determined in a second semi-static study for 21-days (Bruns, 2009) according to OECD 211. Control, solvent control (0.1 mL acetone/L) and the test substance concentrations 12.4, 24.8, 49.6, 99.2, and 198 µg a.s./L were used in the study. 10 Daphnids were used per treatment, 1 daphnia per replicate vessel. Test solutions were renewed every 48 hours (working days) or 72 hours (weekend). Daphnids were fed daily with green algae. Test concentrations were measured on day 0, 12, and 19 (fresh solutions without daphnids) and on days 3, 14, and 21 (aged medium, with daphnids). Analysis were performed by HPLC-UV after dilution with acetonitrile. Behaviour and immobility of the parent daphnids were recorded. First brood and number of offspring was determined. At study termination, body length of the surviving parents was determined. Statistical evaluation was carried out by Barlett's and Kolmogorroff-Smirnov test for homogeneity and normal distribution. Reproduction analysis was performed with ANOVA using Dunnett or Mann-Withney-Wilcoxon. Statistical program used was ToxRat Professional version 2.09.

Results: The mean measured concentrations in the freshly prepared media were 105 – 120% of nominal and in the aged concentrations 99 – 115% of nominal. No effects on parental behaviour were observed. No mortality was determined in any control of test concentration. First brood was at 9.2 days of parent age in the control group, no statistically significant differences were observed for the test concentrations. The cumulative number of offspring per female was 132.0 and 132.8 in the control and solvent control, respectively. The number of offspring in the test concentrations was 127.8, 138.8, 133.6, 134.5, and 120.4 for 12.4, 24.8, 49.6, 99.2, and 198 µg as/L, respectively. No statistically significant difference was found compared to pooled control. Mean body length of parental daphnids was significant reduced at 198 µg as/L at the end of the study compared to control.

The body length of surviving parent daphnids was significantly reduced at the highest test concentration. The overall 21-d NOEC is set at 99.2 µg a.s./L, based on reduction in parental body length and nominal test concentrations.

Remark: Water quality parameters were within acceptable levels. Study is reliable, RI= 1.

5.4.3 Algae and aquatic plants

The toxicity of pencycuron (purity 99.0%) to the green algae *Scenedesmus subspicatus* was tested according to OECD 201 guideline under static conditions (Dorgerloh and Sommer, 2001). Eight nominal test concentrations ranged from 0.1 to 20.0 mg/L were tested together with a control and solvent control (0.1 mL dimethylformamide/L). Six replicates for the control and solvent control and three for each test concentration. Exposure duration was 72 hours. Concentrations of the test substance were analysed from replicate flasks without algae by HPLC. Mean measured concentrations varied between 28% and 103% at the start and between 25 – 109% at the end of the study. The recovery decreased with increasing test concentrations. In the two highest test concentrations undissolved particles were observed, which indicates that the water solubility of 0.3 mg a.s./L was reached. All tested concentrations, except one, were above the water solubility of the test substance of 0.3 mg a.s./L. It must be assumed that the amount of test substance dosed above the aqueous solubility of the active ingredient is not bioavailable and does not contribute to the exposure of the algae to the test substance. As no adverse effects to the algae were observed it can be concluded that under the circumstances of this test, pencycuron is not toxic within its aqueous solubility. 72-h EC50 growth rate is set at > 0.3 mg a.s./L and 72-h NOEC growth rate is ≥ 0.3 mg a.s./L.

Acute toxicity of the degradation product pencycuron-PB-amine to algae.

The toxicity of pencycuron-PB-amine (a degradation product of pencycuron, purity 98.2%) to the green algae *Scenedesmus subspicatus* was tested in a 72-hours static test according to OECD 201 guideline (Seyfried, 2002). Control and the test concentrations 0.22, 0.46, 1.0, 2.2, 4.6, and 10.0 µg/L were used in the study. Six replicates were used for the control and three for each test concentration. Additional flasks were prepared for each concentration and control without algae. Initial cell concentration was 10^4 cells/mL. Actual concentrations were measured the start and at the end of the study of the nominal concentrations 1.0 – 10 µg/L, concentrations 0.22 and 0.46 µg/L were only analysed at the start, since these concentrations were below the 72-h NOEC. Stock solution was also analysed. Each test medium without algae was analysed at the start and end of the test. Analysis by HPLC with MS detection (method recovery 66 – 103%, mean 87%, LOD was 0.071 µg/L). Cell densities were determined using an electronic particle counter. EC-values were established by probit analysis and the NOEC value by Dunnett-test. The recoveries at 0.22 and 0.46 µg/L directly after dosing were very low, i.e 63% due to adsorption of the test substance onto glass surface. Mean measured concentrations (at t= 0 and t= 72 h) at all other test concentrations were > 80% of nominal. The concentrations with very low recoveries were far below the 72-h NOEC value. Mean measured concentrations were 0.14, 0.38, 0.80, 1.75, 3.77, and 8.92 µg as/L for the nominal concentrations 0.22, 0.46, 1.0, 2.2, 4.6, and 10 µg/L, respectively. All test media were clear throughout the test. Cell density in the control after 72 hours was 74 x 10^4 cells/mL (> 16 fold increased). No difference in cell size and shape between test concentrations and control. The 72-h E_bC_{50} and E_rC_{50} were both > 8.92 µg/L, NOE_bC and NOE_rC were 1.75 µg/L and > 8.92 µg/L, all values based on measured test concentrations.

5.4.4 Other aquatic organisms (including sediment)

No adequate data available.

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

Acute aquatic hazards.

No effects on aquatic organisms were observed above the water solubility of 0.3 mg pencycuron/L in fish, Daphnia and algae. Based on the L(E)C50 values of > water solubility of 0.3 mg/L, pencycuron does not fulfil the criteria for classification as acutely toxic to the aquatic environment.

Aquatic Chronic hazards

Pencycuron is considered not rapidly degradable, and it fulfils the criterion for bioaccumulation based on a log Kow ≥ 4. In a chronic toxicity study in fish (OECD 210, Dorgerloh and Sommer, 2002) and in two chronic toxicity studies in *Daphnia magna* (OECD 202, Memmert, 1991; OECD 211, Bruns, 2009), NOEC values of 0.0832 a.s./L (mean measured), 0.067 a.s./L (mean measured) and 0.099 mg a.s./L (nominal) were obtained. Although the study of (Memmert (1991) has some deficiencies, e.g. by being carried out according to guideline OECD202 and having high measured test concentrations, the study is nevertheless considered to be reliable (RI = 2). The results are therefore considered to be acceptable for classification and labelling purposes. The results of the Memmert study are supported by the NOEC of 0.0832 mg/l in fish (Dorgerloh and Sommer, 2001) and the NOEC of 0.099 mg/l in daphnids (Bruns, 2009). The total weight of evidence supports the classification as Aquatic Chronic 1.

The lowest NOEC value is < 0.1 mg/L. Being not rapidly degradable, pencycuron therefore fulfils the criteria for classification as Aquatic Chronic 1. The NOEC value falls within the range 0.01 < NOEC < 0.1 mg/L which results in a chronic M-factor of 1.

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

Table 48

	CLP regulation
Resulting harmonised classification:	Aquatic Chronic 1 (H410: very toxic to aquatic life with long-lasting effects). Chronic M-factor 1

RAC evaluation of environmental hazards
Summary of the Dossier submitter's proposal Pencycuron is currently not listed in Annex VI of CLP. The DS's proposal for harmonised classification and labelling was Aquatic Chronic 1 (H410) with an M-factor of 1. Hydrolytic stability was tested in three studies. The studies showed variable half-life values in the range of 30–289 days at pH 5–9 at environmentally relevant temperatures. Only one of the studies followed the stability of the degradation products and concluded that the only degradation product (pencycuron-PB-amine, M16) was stable at pH 5 and pH 7. No studies on direct photodegradation in water were available. The potential for photodegradation of pencycuron was investigated by testing light absorption at >295 nm. The DS concluded that photodegradation is not expected for pencycuron therefore photolysis test was not performed. Ready biodegradability tests were not reported for pencycuron. However, one

water/sediment simulation study with two types of aerobic water–sediment systems was included in the CLH report. Average DT_{50} values in the simulation tests – for the whole system covering water and sediment – were 139.0 and 82.6 days and therefore the DS concluded that pencycuron is not rapidly degradable in the aquatic environment according to the CLP regulation as it failed to meet the criterion of >70% degradation in 28 days. Mineralization based on radioactivity was 12% and 22% (after 91 days) in the two systems respectively. The metabolite M16 reached 3.5% of the applied radioactivity in water.

One bioaccumulation test was reported but the DS considered it as not reliable. In lack of experimental results the DS concluded that pencycuron fulfils the criterion for bioaccumulative potential, based on a HPLC-shake flask method test which resulted log K_{ow} values of 4.0 at 25 °C and 4.7 at 20 °C.

All acute toxicity studies of pencycuron on fish (four studies reported), invertebrates (one study) and algae (one study) showed relatively low toxicity and did not lead to classification, because the LC_{50} /L_rC_{50} values are estimated to be higher than 0.3 mg/L, which is the water solubility limit of pencycuron.

The degradation product M16 showed low acute toxicity for the tested fish and crustacean species, but the 72-h static, algae test gave an E_rC_{50} >0.00892 mg/L value (highest nominal concentration).

Based on these results the DS proposed no classification for aquatic acute toxicity.

Chronic toxicity of pencycuron was tested in fish (one sub-chronic study), crustacean (two studies) and algae (one study). The lowest observed NOEC values were in the same range for fish and crustaceans: NOEC (94d) = 0.0832 mg/L for *Oncorhynchus mykiss* (based on swim-up and growth, measured concentrations) and NOEC (21d) = 0.067 mg/L for *Daphnia magna* (based on parental immobility and mean measured concentrations). No toxic effects of pencycuron were observed in the algae study.

Based on these results the DS proposed to classify pencycuron as Aquatic Chronic category 1 with an M-factor of 1.

Comments received during public consultation

Three member states agreed with the proposed environmental classification.

One Member State asked for further clarification which was provided by the DS in the RCOM.

One MSCA submitted an additional acute toxicity study on *Daphnia magna*. The study from 1986 showed higher toxicity than the studies included in the report (EC_{50}: 0.20–0.46 mg/L). The DS agreed with the German CA that this study is valid and leads to classification Aquatic Acute 1 with an M-factor 1.

Another MSCA raised a concern about the stabile metabolite M16 and its possible acute toxicity. M16 may be acutely toxic, but not certainly known. since no measured data are available to justify it. On the other hand, new data on Daphnia acute toxicity provided evidence for classification pencycuron as an acutely toxic substance.

One company submitted 10 comments on the bioaccumulation study carried out in fish in 1982. The DS did not take this study into consideration in their original CLH proposal, since it was considered as a non-valid study. The company provided arguments for the acceptable quality of the study, explaining some of the details, which partly were not agreed by, partly not clear to the DS. After these clarifications, the DS accepted the study as valid. Acceptance and inclusion of the fish bioaccumulation study did not impact the CLH proposal, given that classification of chronic toxicity is not dependent on the bioaccumulation potential if adequate long term toxicity and degradation data are present, as is the case for pencycuron.

Additional key elements

Study summary of the acute toxicity study of pencycuron in *Daphnia magna* provided by the German CA during the PC (Heimbach, 1986).

The study was performed according to the OECD TG 202 (1984) and GLP at static conditions. The five pencycuron concentrations applied were 0.032; 0.056; 0.10; 0.18 and 0.32 mg/L. In addition, both water and solvent (acetone 0.1 ml/L) controls were applied. Three replicates (10 animals each) were used for each pencycuron concentration and control. The animals were 6–24 hours old at the beginning of the study. Pencycuron concentrations were not measured during the course of the study and therefore, the reported EC values are based on nominal concentrations. After 24 and 48 hours of exposure, the inability to swim and/or immobility was determined.

No immobility was observed in the water control or at the lowest (0.032 mg/L) pencycuron concentration and one individual (3%) was not able to swim in the solvent control after 48-h exposure. After 24-h of exposure very few animals were affected by pencycuron, however, a clear dose-response was observed in the pencycuron exposed *D. magna* after 48-h exposure: at 0.056 mg/L up to 13% were immobile or unable to swim, at 0.10 mg/l 17%, at 0.18 mg/l 27% and at 0.32 mg/L 67%. As a result the EC_{50}-value (48 h) based on nominal concentrations was determined to be 0.27 mg/L (95% CL 0.20–0.46 mg/L).

Additional details to the 2001 Daphnia study (OECD TG 202, 1984) that was shortly summarised in the CLH report (extracted from the draft assessment report, DAR 2009).

The study from 2001 applied eight different concentrations and the measured concentration recovery for the four lowest concentrations (0.18, 0.32, 0.56 and 1 mg/L) was >80% whereas the recovery rates for the higher concentrations 1.8, 3.2, 5.6 and 10 mg/L were 57%, 30%, 17% and 21%, respectively.

Assessment and comparison with the classification criteria

Degradation

Based on limited hydrolysis, lack of photolysis and a DT_{50} (82.6–139.0 days for the whole system) of >16 days in aerobic water/sediment studies, it can be concluded that pencycuron does not undergo rapid primary degradation in the environment. The maximum mineralization reached was 22% in 91 days in the water/sediment simulation study, so, it failed to meet the criterion of >70% degradation in 28 days. Pencycuron is therefore considered as not rapidly degradable.

The only stable degradation product, pencycuron-PB-amine (M16) reached 3.5% of the applied radioactivity in the water/sediment simulation study and it is considered not to be relevant for classifiction.

Bioaccumulation

The reported bioaccumulation study in carp showed relatively low bioconcentration (BCF = 226 L/kg and 283 L/kg after lipid correction). The DS did not consider the study valid in their original proposal, however, changed the interpretation after PC due to comments that clarified the study details. The reported bioaccumulation study is considered reasonably reliable for classification. Based on the measured fish bioaccumulation in fish pencycuron is considered not to have a potential for bioaccumulation since the BCF is below the CLP criterion of 500 L/kg.

Acute aquatic toxicity

The original proposal by the DS for aquatic acute classification concluded that the observed toxicities in fish, invertebrates, and algae were above the water solubility (0.3 mg/L) limit of pencycuron and no acute classification and labelling for environmental hazards was proposed. RAC agrees that the acute toxicity studies reported in the PC version of the CLH

report do not justify an aquatic acute classification. However, the additional study on invertebrate (*D. magna,* Heimbach, 1986) provided during the PC changed the DS's view and they expressed support to the approach to have aquatic acute classification for pencycuron. The EC_{50} value of 0.27 mg/L of the additional *Daphnia* study from 1986 (OECD TG 202, 1984) was based on nominal concentrations and it does not have any other major weaknesses. In the more recent *Daphnia* study from 2001, test concentrations were measured and all concentrations at and below 1 mg/L showed high recovery rate (>80%). In addition, precipitates were observed at concentrations above 1 mg/L. As there are two nearly equivalent studies available, one of them showing effects and another one not, the reliability of the toxic effects observed in the older study should be evaluated and weighted against the non-observed effects in the 2001 study where the test concentrations were measured and confirmed to be stable throughout the experiment.

In the case of the inclusion of the new *Daphnia* study: acute toxicity of pencycuron measured on *Daphnia*: EC_{50}= 0.20–0.46 mg/L <1 mg/L. It fulfils the criterion for **Aquatic Acute Category 1 with an M-factor of 1.**

Chronic aquatic toxicity
Pencycuron is considered not rapidly degradable, and it does not fulfil the criterion for bioaccumulation. The NOEC (fish) = 0.0832 mg/L and NOEC (crustacea) = 0.067 mg/L are both <0.1 mg/L. These results fulfil the criterion for **Aquatic Chronic Category1** with an **M-factor of 1**.

RAC concludes that pencycuron should be classified as Aquatic Acute 1 (H400) with an M-factor of 1 and as Aquatic Chronic category 1 (H410) with an M-factor of 1.

6 OTHER INFORMATION

This proposal for harmonised classification and labelling is based on the data provided for the registration of pencycuron according to Directive 1107/2009/EEC. The summaries included in this proposal are partly copied from the DAR volume 3, annex B. Some details of the summaries were not included when considered not relevant for a decision on the classification and labelling of this substance. For more details, the reader is referred to the DAR Volume 3 and its addendum.

7 REFERENCES

European Commission. Draft Assessment Report Pencycuron, prepared by The Netherlands, 2009 and update in 2010.

The references to the individual studies are available in the DAR and the update.

8 ANNEXES

None.