Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

picolinafen (ISO); *N*-(4-fluorophenyl)-6-[3-(trifluoromethyl)phenoxy]pyridine-2-carboxamide; 4'-fluoro-6-[(α,α,α-trifluoro-*m*-tolyl)oxy]picolinanilide

EC Number: -
CAS Number: 137641-05-5

CLH-O-0000007040-89-01/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 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

16 September 2021

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification: picolinafen (ISO); *N*-(4-fluorophenyl)-6-[3-(trifluoromethyl)phenoxy]pyridine-2-carboxamide; 4′-fluoro-6-[(*α*,*α*,*α*-trifluoro-*m*-tolyl)oxy]picolinanilide

EC Number: -

CAS Number: **137641-05-5**

Index Number: -

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PICOLINAFEN (ISO); *N*-(4-FLUOROPHENYL)-6-[3-(TRIFLUOROMETHYL)PHENOXY]PYRIDINE-2-CARBOXAMIDE; 4′-FLUORO-6-[(α,α,α-TRIFLUORO-*M*-TOLYL)OXY]PICOLINANILIDE

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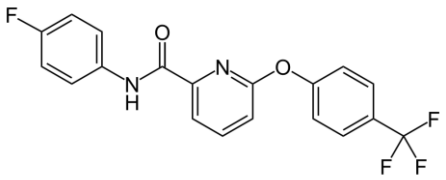
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PICOLINAFEN (ISO); *N*-(4-FLUOROPHENYL)-6-[3-(TRIFLUOROMETHYL)PHENOXY]PYRIDINE-2-CARBOXAMIDE; 4′-FLUORO-6-[(α,α,α-TRIFLUORO-*M*-TOLYL)OXY]PICOLINANILIDE

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	N-(4-fluorophenyl)-6-[3-(trifluoromethyl)phenoxy]pyridine-2-carboxamide; 4′-fluoro-6-[(α,α,α-trifluoro-m-tolyl)oxy]picolinanilide
Other names (usual name, trade name, abbreviation)	Picolinafen
ISO common name (if available and appropriate)	Picolinafen
EC number (if available and appropriate)	n.a.
EC name (if available and appropriate)	n.a.
CAS number (if available)	137641-05-5
Other identity code (if available)	
Molecular formula	$C_{19}H_{12}F_4N_2O_2$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	376.3 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	97 %

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Picolinafen	97.0 % w/w	No entry in Annex VI	GHS09 Wng Aq Acute 1, H400 Aq Chronic 1, H410

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

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

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

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

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

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

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No entry										
Dossier submitters proposal	n.a.	Picolinafen	n.a.	137641-05-5	STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H373 (blood, thyroid) H400 H410	Wng GHS08 GHS09	H373 (blood, thyroid) H410		M=1000 M=1000	
Resulting Annex VI entry if agreed by RAC and COM					STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H373 (blood, thyroid) H400 H410	Wng GHS08 GHS09	H373 (blood, thyroid) H410		M=1000 M=1000	

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

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	*hazard class not assessed in this dossier*	No
Flammable gases (including chemically unstable gases)		
Oxidising gases		
Gases under pressure		
Flammable liquids		
Flammable solids		
Self-reactive substances		
Pyrophoric liquids		
Pyrophoric solids		
Self-heating substances		
Substances which in contact with water emit flammable gases		
Oxidising liquids		
Oxidising solids		
Organic peroxides		
Corrosive to metals		
Acute toxicity via oral route	***data conclusive but not sufficient for classification***	**Yes**
Acute toxicity via dermal route		
Acute toxicity via inhalation route		
Skin corrosion/irritation		
Serious eye damage/eye irritation		
Respiratory sensitisation		
Skin sensitisation		
Germ cell mutagenicity		
Carcinogenicity		
Reproductive toxicity		
Specific target organ toxicity-single exposure		
Specific target organ toxicity-repeated exposure	***harmonised classification proposed***	**Yes**
Aspiration hazard	*criteria not applicable to solids according to Annex 3.10.1.6.2.a*	No
Hazardous to the aquatic environment	***harmonised classification proposed***	**Yes**
Hazardous to the ozone layer	*hazard class not assessed in this dossier*	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Picolinafen is an active substance in the scope of the Regulation (EC) 1107/2009 (repealing Directive 91/414/EEC). The substance is not currently listed in Annex VI of CLP, and there have been no previous classification and labelling discussions of this substance. The substance is therefore subject to the harmonised classification and labelling process in accordance with Article 36(2) of CLP and no further justification is required.

RAC general comment

Picolinafen is an herbicidal active substance in plant protection products (PPP) and therefore it is subject for harmonised classification and labelling (CLH). There is no existing entry in Annex VI of CLP, and there have not been any previous discussions on classification and labelling of picolinafen.

At the time of submission of the CLH report, picolinafen has not been registered under REACH. Under the PPP Regulation (EC) No 1107/2009, picolinafen is subject for renewal procedure and a Renewal Assessment Report has been developed and also the CLH report relies on the same data.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

5 IDENTIFIED USES

Picolinafen is an active substance in plant protection products with uses as an herbicide.

6 DATA SOURCES

Main data source for this CLH dossier are Volumes 1 and 3 of the Renewal Assessment Report (RAR) which was prepared for the pesticides procedure.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	solid	Werle, 1997	measured
Melting/freezing point	Melting range of 107.2 - 107.6 °C	Mangels, 1996	measured
Boiling point	No defined boiling point observable, decomposition at > 230 °C	Werle, 1996	measured
Relative density	d_4^{20}: 1.45 g/cm^3	Werle, 1997	measured

Property	Value	Reference	Comment (e.g. measured or estimated)
Vapour pressure	$2.4\pm1.0 \cdot 10^{-4}$ Pa (70 °C) $8.5\pm4.2 \cdot 10^{-4}$ Pa (80 °C) $2.4\pm1.1 \cdot 10^{-3}$ Pa (90 °C) extrapolated values: $1.7 \cdot 10^{-7}$ Pa (20 °C) $3.8 \cdot 10^{-7}$ Pa (25 °C)	Madsen and An, 1997	measured
Surface tension	An aqueous solution of the test material has a surface tension of 72.3 mN/m	Werle, 1997	measured
Water solubility	at 20 °C: pH 5 buffer: 3.8 · 10-5 g/l pH 7 buffer: 4.7 · 10-5 g/l pH 9 buffer: 3.8 · 10-5 g/l DI water: 3.9 · 10-5 g/l at 10 °C: DI water: 3.0 · 10-5 g/l at 30 °C: DI water: 6.8 · 10-5 g/l	Kuhn, 1996	measured
Partition coefficient n-octanol/water	Solvent log POW DI water 5.37 pH 5 buffer 5.36 pH 7 buffer 5.43 pH 9 buffer 5.36	Coover, 1996	measured
Granulometry	fine crystalline solid, forms small globular agglomerates of ca. 2 mm diameter	Werle, 1997	measured
Stability in organic solvents and identity of relevant degradation products	PAS at 20 °C: acetone: 236 g/l dichloromethane: 561 g/l ethyl acetate: 227 g/l n-hexane: 3.87 g/l methanol: 28.4 g/l toluene: 222 g/l TAS at 20 °C: acetone: 557 g/l dichloromethane: 764 g/l ethyl acetate: 464 g/l n-hexane: 3.8 g/l methanol: 30.4 g/l toluene: 263 g/l	Kuhn, 1996 Holman, 1998	measured
Dissociation constant	Preliminary tests using spectrophotometric and titration methods indicated that Picolinafen does not dissociate in the pH range of 2 – 12.	Holman, 1997	measured
Viscosity	Not applicable	-	The substance is a solid

8 EVALUATION OF PHYSICAL HAZARDS

Not addressed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of toxicokinetic studies

Method	Results	Reference
Absorption, Distribution, Metabolism and Excretion of [14C]Picolinafen in rats OECD TG 417 (1984) GLP	**Absorption:** About 60 % were absorbed within 48 h, based on urinary (16-70 %) and biliary (8-34 %) excretion with considerable differences between sexes and position of the label in the molecule. Only low amounts of compound were exhaled via air. Administration of higher doses leads to higher tissue concentrations in the evaluated tissues. **Distribution:** Wide distribution between tissues was reported, highest concentrations of residues were observed in blood, liver, and kidney. **Metabolism:** moderately metabolised in rats (cleavage of amide bond, oxidation and conjugation) **Excretion:** almost completely excreted within 48 hours (86-89 % of the single low dose)	Anonymous 1, 1999

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

The available study included a pilot experiment, a definitive experiment with 10 groups of male and female Sprague-Dawley rats (Crl: CD BR for single and multiple dose groups; Crl: CVF for biliary excretion groups) fed *ad libitum* and treated with Picolinafen radiolabelled at two different positions (i.e. [^{14}C]pyridine label or [^{14}C]aniline label) and a supplemental experiment with male rats only. Nominal dosages were 10 mg/kg bw as the low dose and 1000 mg/kg bw as the high dose.

The study showed that Picolinafen orally administered to rats was readily absorbed. The absorption in animals of the bile-cannulated groups receiving the lower dose within 48 hours was approximately 51 % (male) and 67 % (female) for the [^{14}C]pyridine label, and 60 % (male) and 84 % (female) for the [^{14}C]aniline label. For the bile-cannulated groups receiving the higher dose, the percent absorption decreased to 17 % and 25 % for the aniline and pyridine label, respectively - presumably due to saturation of absorption, as 31-65 % of the administered dose was still present in the gastrointestinal contents.

Picolinafen was almost completely excreted within 48 hours (86-89 % of the single low dose). Males excreted significantly more pyridine-related residue in faeces (~68 %) than in urine (~20 %) and comparable amounts of aniline-related radioactivity in faeces (~40 %) and in urine (~48 %), whereas females eliminated a greater amount of aniline-derived radioactivity in urine (~62 %) than in faeces (~25 %) and comparable amounts of pyridine-derived radioactivity in faeces (~47 %) and in urine (~39 %).

Within 48 hours, 25-34 % and 8-12 % of the administered low dose was excreted in bile of rats treated with pyridine- and aniline-labelled Picolinafen, respectively. In the same period, animals from the high dose group (treated with pyridine-labelled Picolinafen) eliminated 12 % (female) and 17 % (male) of the administered dose in bile and animals from the high dose group (treated with aniline-labelled Picolinafen) eliminated 2 % (both male and female) of the administered dose in bile. Overall recovery of radioactivity

from the biliary study ranged from 93-99 %. Animals in the multiple low dose experiments excreted 90-96 % of the administered dose in urine and faeces within 24 hours after 7 consecutive days of dosing.

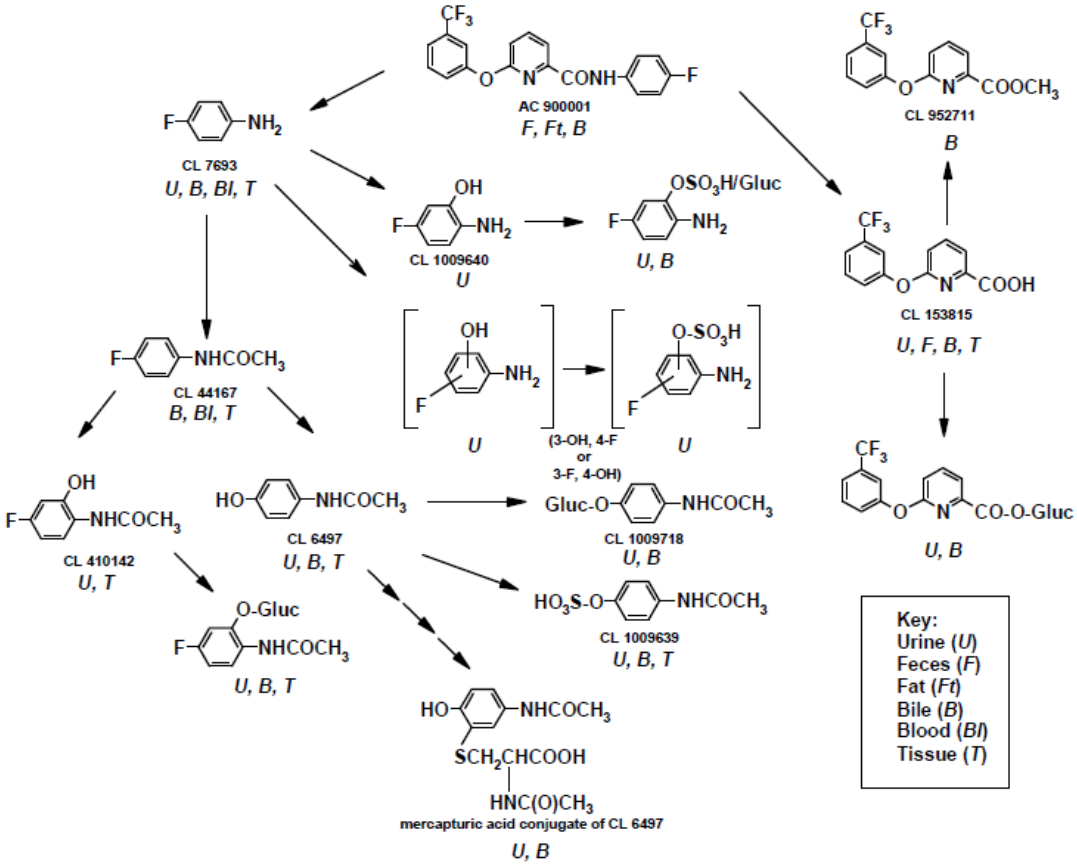
There was no evidence of a potential for bioaccumulation. Less than 0.5 % of the administered dose was detected in the tissues and carcass by 7 days post-dosing. Tissue residue values ranged from 0.004-2.513 ppm in the low dose group and from 0.268-23.005 ppm in the high dose group. The tissues with the highest concentrations were fat, liver and kidneys in rats treated with pyridine labelled Picolinafen and blood, spleen and liver in rats treated with aniline labelled Picolinafen.

Based on the major metabolites that were identified in rat urine, faeces, bile, and specific tissues, a metabolic pathway was proposed involving hydrolysis, oxidation, acetylation, and subsequent glucuronide and sulfate conjugations as major biotransformation processes for Picolinafen in the rat.

Irrespective of the label, Picolinafen was the predominant radio component in faeces, accounting for 97-99 % of the extractable radioactivity. In urine and bile, the substituted picolinic acid CL 153815 and its glucuronide ester were the major metabolites (58.2-84.1 % and 7.2-29.2 %, respectively) when the [pyridine-^{14}C]-labelled Picolinafen was administered, whereas a more complex metabolic profile was obtained with the [aniline-^{14}C]-labelled Picolinafen. This included the sulphate conjugates of 2-amino-5-fluorophenol and acetaminophen (52.9 and 26.1 %, respectively), the mercapturic acid conjugate of acetaminophen (9.1 %), acetaminophen itself (3.4 %), the glucuronide conjugate of acetaminophen (2.7 %) and the sulphate conjugate of 5-amino-2-fluorophenol (2.6 %), plus several minor metabolites accounting to less than 5 % of the total urinary radioactivity (including p-fluoroaniline CL 7693, 2-amino-5-fluorophenol, 4'-fluoro-2'hydroxyacetanilide CL410142, and several minor unknown). A trace amount of parent Picolinafen (0.4 %) was also detected in the bile from aniline-label treated rats.

The proposed metabolic pathway is presented in Figure 1.

Figure 1 Metabolic pathway of Picolinafen in rats as described in the study report



10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Picolinafen proved to be of low acute oral toxicity in rats. One study was submitted which is presented in the following table.

Table 10: Summary table of animal study on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Oral (single gavage) OECD TG 401	Sprague Dawley rat Crl:CD(SD)BR 5 F, 5 M	Picolinafen technical (Batch CA14113; 97.8 % as) as a 25 % w/v dispersion in carboxymethyl cellulose	5000 mg/kg bw/d (dose volume of 20 ml/kg bw)	>5000	Anonymous 5, 1997

According to the notifier, there have been no reports of illness or adverse health effects for any of the employees working in the plant or in the medical department of one production site in Langenfeld, Germany. No other information is available.

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

No treatment-related mortality or clinical signs of toxicity were observed during the 14-day study period after exposure to 5000 mg/kg bw of the substance.

10.1.2 Comparison with the CLP criteria

Table 11 presents the results of the valid toxicological study in comparison with the CLP criteria for acute oral toxicity. The oral LD50 value exceeded the highest dose of 2000 mg/kg bodyweight for classifying acute toxicity hazard categories.

Table 11: Results of acute oral toxicity in comparison with CLP criteria

Result of the toxicological study	CLP criteria
LD_{50} > 5000 mg/kg (oral, gavage) in rat	Cat 4 (H302): 300 < LD_{50} ≤ 2000 mg/kg (oral) Cat. 3 (H301): 50 < LD_{50} ≤ 300 mg/kg (oral) Cat. 2 (H300): 5 < LD_{50} ≤ 50 mg/kg (oral) Cat. 1 (H300): LD_{50} ≤ 5 mg/kg (oral)

10.1.3 Conclusion on classification and labelling for acute oral toxicity

The valid study on acute toxicity does not support classification and labelling of Picolinafen for this endpoint. Likewise, there is no human information pointing to such a need. Therefore, Picolinafen should not be classified for acute oral toxicity.

10.2 Acute toxicity - dermal route

Picolinafen proved to be of low acute dermal toxicity in rats. One study was submitted, which is presented in the following table.

Table 12: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LD_{50}	Reference
Dermal OECD TG 402 GLP	Sprague Dawley rat 5 M, 5 F	Picolinafen technical (Batch CA14113; 97.8 % as)	4000 mg/kg bw, 24 hours exposure period	> 4000 mg/kg bw	Anonymous 4, 1997

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

No mortality or macroscopic pathological changes were observed during or after the 14 day study period after topical application of 4000 mg/kg bw. However, body weight loss of 4 grams was observed in one female at 4000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

Table 13 presents the results of the valid toxicological study in comparison with the CLP criteria for acute dermal toxicity. The dermal LD_{50} value was above 2000 mg/kg bw for classifying acute toxicity hazard categories.

Table 13: Results of acute dermal toxicity in comparison with CLP criteria

Result of the toxicological studies	CLP criteria
LD_{50} > 4000 mg/kg (dermal) in rat	Cat. 4 (H312): 1000 < LD_{50} ≤ 2000 mg/kg (dermal) Cat. 3 (H311): 200 < LD_{50} ≤ 1000 mg/kg (dermal) Cat. 2 (H310): 50 < LD_{50} ≤ 200 mg/kg (dermal) Cat. 1 (H310): LD_{50} ≤ 50 mg/kg (dermal)

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the available evidence, Picolinafen should not be classified for acute dermal toxicity.

10.3 Acute toxicity - inhalation route

Picolinafen proved to be of low acute inhalation toxicity in rats. The submitted study is shown in the following table.

Table 14: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, , form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** LC_{50}	**Reference**
Inhalation (nose-only) OECD TG 403 GLP	Sprague Dawley rat 5 M, 5 F	Picolinafen technical (Batch CA14113; 97.8 % as) administered as a dust (milled prior to administration) MMAD: 5.8 microns with a geometric standard deviation of 1.6 microns	4 hours via nose-only inhalation	>5.9 mg/L	Anonymous 7, 1997

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

In the acute inhalation toxicity study (Anonymous 7, 1997), no mortality occurred. Labored breathing was noted during the 4-hour exposure period. Labored breathing, moist rales, clear nasal discharge, salivation and chromodacryorrhea were observed during the first 2 hours following exposure for animals exposed to Picolinafen technical. These responses continued during the first two days following exposure (study days 2 and 3) and were resolved for all animals by study day 4. All animals gained weight during the 14-day post-exposure observation period, and no macroscopic findings were noted at necropsy.

10.3.2 Comparison with the CLP criteria

Table 15 presents the results of the valid toxicological study in comparison with the CLP criteria for acute inhalation toxicity. The inhalation LD50 was above 5.9 mg/L and hence above the highest reference dose of 5.0 mg/L for classifying acute toxicity hazard categories.

Table 15: Results of acute inhalation toxicity in comparison with CLP criteria

Result of the toxicological study	**CLP criteria**
LD_{50} > 5.9 mg/L (inhalation) in rat	Cat. 4 (H332): 1.0 < LC_{50} ≤ 5.0 mg/L (dusts and mists) Cat. 3 (H331): 0.5 < LC_{50} ≤ 1.0 mg/L (dusts and mists) Cat. 2 (H330): 0.05< LC_{50} ≤ 0.5 mg/L (dusts and mists) Cat. 1 (H330): LC_{50} ≤ 0.05 mg/L (dusts and mists)

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the available evidence, Picolinafen should not be classified for acute inhalation toxicity.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of picolinafen for acute oral toxicity based on one negative study performed with 5 females and 5 males Sprague Dawley rats according to GLP and OECD TG 401 (Anonymous 5, 1997). The estimated LD_{50} was > 5000 mg/kg bw.

The DS proposed no classification of picolinafen for acute dermal toxicity based on no mortality and adverse toxic effects in the GLP and OECD TG 402 study performed with 5 females and 5 males Sprague Dawley rats exposed topically for 24 hours to 4000 mg /kg bw followed by a 14-day observation period (Anonymous 4, 1997). The estimated LD_{50} was > 4000 mg/kg bw.

The DS proposed no classification for acute inhalation toxicity. In an OECD TG 403 acute inhalation study (Anonymous 7, 1997) performed in GLP conditions, 5 male and 5 female Sprague Dawley rats were exposed (nose-only) for 4h to a dust aerosol of picolinafen technical at a concentration of 5.9 mg/L. The particle size of the tested dust was 5.8 µm MMAD with a geometric standard deviation of 1.6 microns. No mortality was observed. Laboured breathing was noted during the 4-hour exposure period. Also, moist rales, clear nasal discharge, salivation and red lacrimal secretion (chromodacryorrhea) were observed during the first 2 hours following exposure to picolinafen technical. These responses continued during the first two days following exposure (study days 2 and 3) and were resolved for all animals by day 4. All animals gained weight during the 14-day post-exposure observation period, and no macroscopic findings were noted at necropsy. The estimated LC_{50} was > 5.9 mg/L.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Noting that the oral LD_{50} for rats is > 5000 mg/kg bw, which is above the upper limit value of 2000 mg/kg bw for classification in category 4, picolinafen does not warrant classification for acute oral toxicity.

Taking into account that the dermal LD_{50} for rats is > 4000 mg/kg bw, which is above the upper limit value of 2000 mg/kg bw for classification in category 4, picolinafen does not warrant classification for acute dermal toxicity.

Since the LC_{50} for rats is > 5.9 mg/L , which is above the upper limit value for dust and mist for classification in category 4, picolinafen does not warrant classification for acute inhalation toxicity.

10.4 Skin corrosion/irritation

Table 16: Summary table of animal study on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Dermal Irritation Study OECD TG 404	New Zealand White rabbit 6 M	Picolinafen technical (lot: CA14113, purity: 97.8 % as	0.5 g moistened with 0.5 of distilled water 4 hours exposure period	No erythema or edema in any animal at any time point	Anonymous 2, 1997

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

One study was submitted (Anonymous 2, 1997) in which no signs of skin irritation or corrosion were observed in any animal.

10.4.2 Comparison with the CLP criteria

Table 17 presents the results of the valid toxicological study in comparison with the CLP criteria for skin irritation and corrosion.

Table 17: Results of skin irritation study in comparison with CLP criteria

Result of the toxicological study	CLP criteria
Mean erythema and oedema scores (24-72 h): 0.0 and 0.0, respectively (no animal ≥ 0)	Irritating to skin (Category 2, H315): at least in 2/3 tested animal a positive response of: Mean value of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Based on the available evidence, the substance does not meet the criteria for classification for skin corrosion or irritation.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS presented results of the dermal irritation study (GLP, OECD TG 404, Anonymous 2, 1997) showing that no erythema or oedema at any time point were observed in any of 6 adult male New Zealand White rabbits exposed to 0.5 g picolinafen moistened with 0.5 mL of distilled water, applied to the intact shaved flank under a semi-occlusive dressing, for 4 hours. No clinical signs were observed in the animals during the study and no mortality occurred. No corrosive effects were noted on the treated skin of any animal at any of the observation intervals. The irritation scores for erythema and oedema at 24, 48

and 72 hours for all animals was 0.0. Based on the available evidence the DS concluded that the substance does not meet the criteria for classification for skin corrosion or irritation.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Taking into account the evidence from the reliable dermal irritation study in rabbits showing that no skin reaction was observed in any of the treated animals (mean scores for erythema and oedema equal 0.0 for all animals), RAC is of the opinion that picolinafen does not warrant classification for skin irritation/corrosion.

10.5 Serious eye damage/eye irritation

Table 18: Summary table of animal study on serious eye damage/eye irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels duration of exposure</th><th>Results
- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr><td>Primary Eye Irritation
OECD TG 405
GLP</td><td>New Zealand White Rabbit
6 M</td><td>Picolinafen technical (Batch CA14113; 97.8 % as)</td><td>0.1 mL
24 hours exposure period</td><td>Slight conjunctival redness for all 6 rabbits at 1 hour (scores of 1) and conjunctival discharge in 2 rabbits at 1 hours, in 1 rabbit at 24 hours
All findings reversible after 48 hours
<table>
<tr><td></td><td colspan="6">Average scores for 24-, 48-, and 72 hour Observations</td><td></td></tr>
<tr><td>Area observed</td><td>Male no 61</td><td>Male no 63</td><td>Male no 68</td><td>Male no 74</td><td>Male no 81</td><td>Male no 82</td><td>Overall average score</td></tr>
<tr><td>Corneal opacity</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td></tr>
<tr><td>Iris effects</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td></tr>
<tr><td>Conjunctivae redness</td><td>0.0</td><td>0.0</td><td>0.3</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.05</td></tr>
<tr><td>Conjuctivae</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.0</td></tr>
</table></td><td>Anonymous 3, 1997</td></tr>
</table>

				chemosis								

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

The available valid toxicological study (Anonymous 3, 1997) showed no potential for serious eye damage or eye irritation in New Zealand White Rabbits. No signs of corneal irritation or iris effects were observed in any of the test animals. Conjunctival redness was present in all test animals 1 hours after installation of the substance into the conjunctival sac and persisted in one test animal at the 24 hours observation. Conjunctival discharge was seen in two animals 1 hour after instillation and in one animal at 24 hours. All effects were reversible at 48 hours after installation of the substance.

10.5.2 Comparison with the CLP criteria

Table 19 presents the results of the valid toxicological study in comparison with the CLP criteria for eye irritation.

Table 19: Results of the eye irritation study in comparison with the CLP criteria

Result of the toxicological study	CLP criteria
Mean score (24-72 h): corneal opacity: no animal ≥ 1 iris lesion: no animal ≥ 1 conjunctival redness: no animal ≥ 2 oedema of the conjunctivae (chemosis): no animal ≥ 2	Irritating to eyes (Category 2, H319): at least in 2/3 tested animal a positive response of: corneal opacity: ≥ 1 and/or iritis: ≥ 1 and/or conjunctival redness: ≥ 2 and/or conjunctival oedema (chemosis): ≥ 2

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Based on the available evidence, Picolinafen should not be classified for serious eye damage or eye irritation.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

In the eye irritation study performed according to OECD TG 405 and GLP conditions (Anonymous 3, 1997), at 1 hour after instillation of 0.1 mL of technical picolinafen into conjunctival sac of 6 male rabbits, a slight conjunctival redness was noted in all animals (scores of 1). The conjunctival discharge was observed in 2 rabbits at 1 hour and in 1 rabbit after instillation at 24 hours. All these findings were reversible after 48 hours. The irritation scores for corneal opacity, iris effects, conjunctivae redness and conjunctivae chemosis at 24, 48, and 72 hour observations in all animals was 0.0. No clinical signs of systemic toxicity were observed in the animals during the study.

The DS did not propose classification of picolinafen for serious eye damage or eye

irritation.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Taking into account the evidence from the reliable eye irritation study in rabbits showing that no significant eye reaction in any of the treated animals was observed (mean scores for corneal opacity, iris effects, conjunctivae redness and conjunctivae chemosis were 0.0), RAC is of the opinion that picolinafen does not warrant classification for serious eye damage or eye irritation.

10.6 Respiratory sensitisation

This endpoint is not addressed in this CLH dossier.

10.7 Skin sensitisation

Table 20: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Maximization Test OECD TG 406 GLP	Guinea Pigs Crl:(HA)BR strain 4 M	Picolinafen technical (Batch CA14113; 97.8 % as)	5 % w/v mixture in 0.5 % CMC in distilled water and FCA for intradermal injection and 25 % w/w mixture in petrolatum for topical induction application and for challenge phase Exposure period 24 hours	Scab formation and mild to moderate erythema and edema at intradermal and topical induction application sites. No dermal reaction to the challenge application at 24 and 48 hour observation.	Anonymous 6, 1997

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

In the valid skin sensitisation study (Anonymous 6, 1997), the animals exhibited scab formation and mild to moderate erythema and edema at the intradermal and topical induction application sites. None of the guinea pigs exhibited a dermal reaction to the challenge application of the substance.

10.7.2 Comparison with the CLP criteria

Table 21 presents the results of the skin sensitisation study in comparison with CLP criteria for skin sensitisation.

Table 21: Results of skin sensitisation in comparison with CLP criteria

Result of the toxicological study	CLP criteria
0/20 animals positive 5 % intra dermal induction concentration	Guinea pig maximisation test Category 1A (H317): ≥ 30 % responding at ≤ 0.1 % intradermal induction dose or ≥ 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose Category 1B (H317): ≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose
No non-adjuvant type study submitted	Buehler assay Category 1A (H317): ≥ 15 % responding at ≤ 0.2 % topical induction dose or ≥ 60 % responding at > 0.2 % to ≤ 20 % topical induction dose Category 1B (H317): ≥ 15 % to < 60 % responding at > 0.2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the available evidence, Picolinafen should not be classified for skin sensitisation.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Skin sensitisation potential was assessed in the Guinea Pig Maximization Test (OECD TG 406) and in GLP conditions. A 5% w/v mixture of technical picolinafen in 0.5% carboxymethyl cellulosa in distilled water and a Freund's complete adjuvant was used for intradermal injection. A 25% w/w mixture of technical picolinafen in petrolatum was used for topical induction application as well as for challenge phase. The animals exhibited scab formation and mild to moderate erythema and oedema at the intradermal and topical induction application sites. None of the guinea pigs exhibited a dermal reaction at 24 and 48 hour observation to the challenge application of the substance.

The DS did not propose classification for skin sensitisation.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

No information is provided on the procedure used for selection of the concentrations of the substance for intradermal and topical induction in the study (Anonymous 6, 1997). However, it is reported that scab formation and mild to moderate erythema and oedema of skin were observed at the intradermal and topical induction indicating that concentrations used were causing mild-to-moderate skin irritation. As no skin reactions were observed after the challenge, RAC concludes that picolinafen does not warrant classification for skin sensitisation.

10.8 Germ cell mutagenicity

Picolinafen was tested in a battery of in vitro and in vivo mutagenicity assays measuring several different end points of potential mutagenicity such as gene mutation in bacteria, gene mutation in mammalian cells, and chromosomal aberration in somatic cells. The results are summarised in Table 22 and Table 23.

Table 22: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial/Microsome Mutagenicity Assay OECD TG 471 and 472 GLP supplementary	Picolinafen technical (Batch CA14113; 97.8 % as)	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538; *E. coli* WP2 uvrA- Concentrations: 100, 250, 500, 1000, 2500 µg/plate Dose levels selected for the definitive assay were based on results from a range-finding study conducted at 250, 500, 2,500 or 5,000 µg/plate Tested in the presence and absence of metabolic activation	**Negative +/- S9** Positive controls gave expected results Non mutagenic in tested strains	American Cyanamid Company 1997e
Mammalian Cell CHO/HGPRT Mutagenicity Assay OECD TG 476 GLP supplementary	Picolinafen technical (Batch CA14113; 97.8 % as)	Chinese Hamster Ovary (CHO) cells Concentrations were chosen based on the toxicity results (10, 25, 50, 100, 200 and 300 µg/mL) Tested in the presence and absence of metabolic activation	**Negative +/- S9** Positive control gave expected results Non mutagenic in tested CHO cells (+/-S9 mix)	MA BioServices 1997
In Vitro Chromosome Aberration Assay OECD TG 473 GLP	Picolinafen technical (Batch CA14113; 97.8 % as)	Chinese Hamster ovary (CHO) cells Concentrations: + S9 : 10, 25, 50, 100, 200, 300, 400, 600 µg/mL -S9 : 10, 25, 50, 100, 200, 400,	**Negative +/- S9** Positive control gave expected results No significant increase in chromosome aberration	American Cyanamid company 1997f

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
acceptable		600, 800, 1000 µg/mL		

Table 23: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
In vivo Micronucleus Assay OECD TG 474 GLP acceptable	Picolinafen technical (Batch CA14113; 97.8 % as)	Mouse, Crl:CD-1 (ICR) BR 6 M for 500 mg/kg bw 6 M for 1000 mg/kg bw 12 M for 2000 mg/kg bw Single oral gavage, in 0.5 % (w/v) carboxymethylcellulose Positive control: 80 mg/kg bw cyclophosphamide Bone marrow smears were used for counting of polychromatic erythrocytes and the incidence of micronuclei	Negative Positive control gave expected results. **No clinical signs of toxicity were noted in the treated animals. PCE:NCE ratio was not altered.** Test material did not induce micronuclei in bone marrow.	Anonymous 16, 1999

No human data are available.

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

Results from the *in vitro* and *in vivo* studies indicate that Picolinafen does not induce base pair or frame-shift mutation in any of the bacterial tester strains, or gene mutation in mammalian cells in culture. No potential for clastogenicity was observed in the submitted *in vitro* chromosomal aberration assay in CHO cells or in the *in vivo* mouse micronucleus assay when tested up to the limit dose. However, no indication of toxicity in the target tissue nor any clinical signs of toxicity were reported.

10.8.2 Comparison with the CLP criteria

Comparison with criteria for classification and labelling and conclusion

Following criteria for classification for gem cell mutagens are given in CLP regulation:

CLP regulation
The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans. The classification in Category 1B is based on: — positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or — positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or — positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people. The classification in Category 2 is based on: — positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: — somatic cell mutagenicity tests *in vivo*, in mammals; or — other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

No human data are available for Picolinafen; hence, a classification in category 1A is not possible. Neither *in vivo* heritable germ cell mutagenicity tests nor positive results from *in vivo* somatic cell mutagenicity tests in mammals are available; hence, a classification in 1B is not possible. *In vitro* studies (mutagenicity, clastogenicity) and the respective *in vivo* study showed a negative outcome, hence a classification in category 2 is considered not necessary.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on the negative in vivo studies, no classification is proposed for germ cell mutagenicity.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS reported that picolinafen was tested in a range of *in vitro* and *in vivo* genotoxicity assays.

Table: *Summary of genotoxicity tests with picolinafen (adapted from table 22 and 23 in the CLH report)*

Study	Result	Test System	Reference
***In vitro* studies**			
Bacterial/Microsome Mutagenicity Assay GLP, OECD TG 471 and 472	negative	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538; *E. coli* WP2 uvrA Concentrations chosen based on the toxicity results: 100, 250, 500, 1000, 2500 µg/plate Tested in the presence and absence of metabolic activation	American Cyanamid Company (1997e)
Mammalian cell mutagenicity assay GLP, OECD TG 476 Mammalian Cell CHO/HGPRT Mutagenicity Assay Chinese Hamster Ovary (CHO) cells	negative	Concentrations chosen based on the toxicity results: 10, 25, 50, 100, 200 and 300 µg/mL Tested in the presence and absence of metabolic activation	MA BioServices (1997)
Clastogenicity assay GLP, OECD TG 473 *In vitro* Chromosome Aberration Assay Chinese Hamster ovary (CHO) cells	negative	Concentrations: + S9: 10, 25, 50, 100, 200, 300, 400, 600 µg/mL - S9: 10, 25, 50, 100, 200, 400, 600, 800, 1000 µg/mL	American Cyanamid company (1997f)
***In vivo* studies**			
Micronucleus assay GLP, OECD TG 474 Male NMRI mouse bone marrow (short term)	negative	Concentrations: 6 M for 500 mg/kg bw 6 M for 1000 mg/kg bw 12 M for 2000 mg/kg bw Single oral gavage, in 0.5 % (w/v) carboxymethylcellulose	Anonymous 16, 1999

No human data are available.

***In vitro* studies**

Picolinafen was found to be negative in two reverse mutagenicity tests in bacteria with and without metabolic activation. In the mutagenicity studies in Chinese Hamster ovary cells picolinafen did not increase in the presence or absence of S9-mix the frequency of forward mutations in reporter genes or the frequency of structural chromosomal aberrations.

***In vivo* studies**

In vivo micronucleus assay in mice with picolinafen did not induce micronuclei in the polychromatic erythrocytes of the bone marrow. No clinical signs of toxicity were noted in the treated animals. Proportion of immature erythrocytes among total erythrocytes (PCE:NCE) ratio was not altered, indicating that picolinafen did not have any significant

cytotoxicity in the bone marrow.

There were no studies in germ cells.

The DS did not propose to classify picolinafen as mutagenic.

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

Taking into account negative results obtained in several *in vitro* mutagenicity studies and in one *in vivo* micronucleus assay, RAC considers that picolinafen does not warrant classification for germ cell mutagenicity.

10.9 Carcinogenicity

One supplementary 24-months study in rats and one acceptable 18-months study in mice were submitted. The results are summarized in Table 24.

Table 24: Summary table of animal studies on carcinogenicity

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>24-month study in rats
OECD TG 453
GLP
Supplementary (survival at 24 months: 24 - 31 % for males, 33 % - 43 % for females)
Sprague Dawley rats Crl: CD® (SD) BR
65 M + 65 F per group</td><td>Picolinafen technical (Batch CA14113; 97.8 % as)
2.4/3.0, 12.1/15.0, 24.5/31.0 mg/kg bw/d for m/f
24 months</td><td>Neoplastic effects: At the top dose, increased incidence of benign and malignant neoplasms in the adrenal gland (medulla) in males
<table>
<tr><th>Dose group (ppm)</th><th>0</th><th>50</th><th>250</th><th>500</th></tr>
<tr><td>12-mo interim sacrifice</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Animals examined</td><td>10</td><td>0</td><td>1</td><td>10</td></tr>
<tr><td>Medulla: benign neoplasm (unilateral)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Medulla: benign neoplasm (bilateral)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Medulla: malignant neoplasm (unilateral)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Unscheduled Deaths</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Animals examined</td><td>42</td><td>40</td><td>39</td><td>40</td></tr>
<tr><td>Medulla: benign neoplasm (unilateral)</td><td>3</td><td>5</td><td>2</td><td>4</td></tr>
<tr><td>Medulla: benign neoplasm (bilateral)</td><td>1</td><td>1</td><td>0</td><td>0</td></tr>
<tr><td>Medulla: malignant neoplasm (unilateral)</td><td>0</td><td>1</td><td>1</td><td>1*</td></tr>
<tr><td>Terminal sacrifice</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Animals examined</td><td>13</td><td>2</td><td>3</td><td>15</td></tr>
<tr><td>Medulla: benign neoplasm (unilateral)</td><td>2</td><td>0</td><td>2</td><td>4</td></tr>
<tr><td>Medulla: benign neoplasm (bilateral)</td><td>0</td><td>1</td><td>0</td><td>3</td></tr>
<tr><td>Medulla: malignant neoplasm (unilateral)</td><td>0</td><td>1</td><td>1</td><td>1</td></tr>
<tr><td>All animals</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Animals examined</td><td>65</td><td>42</td><td>43</td><td>65</td></tr>
<tr><td>Medulla: benign neoplasm (unilateral)</td><td>5</td><td>5</td><td>5</td><td>8</td></tr>
<tr><td>Medulla: benign neoplasm (bilateral)</td><td>1</td><td>2</td><td>0</td><td>3</td></tr>
<tr><td>Medulla: malignant neoplasm (unilateral)</td><td>0</td><td>2</td><td>2</td><td>1</td></tr>
</table>
*Metastasis from lympho-reticular system
Non-neoplastic effects at 250 ppm (12.1 mg/kg bw/d): Anaemia (decreased red blood cell parameters); spleen (increased weights, haemosiderin)</td><td>Anonymous 18, 1999</td></tr>
<tr><td>18-months study in mice
OECD TG</td><td>Picolinafen technical (Batch CA14113;</td><td>No treatment-related increase in the type or incidence of tumours
Haematology (increased reticulocyte counts and MCHC); liver (increased weights, hypertrophy); spleen pigment</td><td>Anonymous 17, 1999</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
451 GLP Acceptable CD®-1 albino mice 65 M + 65 F per group	97.8 % as) 0, 6.9/8.2, 68.6/81.0, 137.1/165.8 mg/kg bw/d for m/f 18 months		

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

In the 24-months rat study (Anonymous 18, 1999), a numerical increase, which was not statistically significant, in the incidence of benign neoplasms in the adrenal gland (medulla) was seen in males. The incidence of benign medullary neoplasms for males at 500 ppm (= 24.5/31.0 mg/kg bw/d for m/f) (17 %) is above the range of HCD (5 studies submitted: 0 %, 0 %, 9.8 %, 0 %, 5 % for unilateral benign neoplasms in medulla and 0 %, 0 %, 3.9 %, 0 % and 1.6 % for bilateral benign neoplasms in medulla). HCD were generated in the same laboratory from 5 studies, which were performed between 1991 and 1995. The applicant also submitted further HCD, which did not fulfil the quality criteria, as they were collected between 1984 and 1989. The applicant further stated that "*the incidence of malignant medullary neoplasms of 1/65 (2 %) for males at 500 ppm in the study with Picolinafen is actually the minimal incidence rate for this historical control database. In addition, Picolinafen did not shorten the latency to this tumor type and did not induce a dose-dependent increase in the incidences of preneoplastic changes (hyperplasia/basophilia).*"

The quality of this study was adversely affected by a reduced survival rate of the animals. Survival rates were 24 %, 29 %, 31 % and 29 % for males and 42 %, 43 %, 33 % and 35 % for females (for control group, 50, 250 and 500 ppm groups, respectively). The notifier submitted the following information:

"*There were some deviations to OECD guideline 453 in the 24-month rat study: (i) number of animals of the high dose satellite group (10-15 instead of the 20 required); (ii) number of animals evaluated for haematology at each time point (10 instead of 20) and (iii) survival to the end of the study. However, the study was considered acceptable, based on acceptability under US EPA guideline criteria, EEC position against needless repetition of tests on animals and historical control data from the performing laboratory evidencing the survival rate of control males in the study being within recent historical control range. Although parameters were obtained from a number of animals lower than that required, the results of the study are clear and correctly identified critical effects. In addition, the dog and not the rat was identified as the most sensitive species and subsequent ADI and AOEL values were based on the dog studies. For these reasons, it is not considered useful or necessary to repeat this study.*"

In the mouse study (Anonymous 17, 1999), no treatment-related increase in type or incidence of tumours was seen.

Table 25: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Sprague Dawley rats Crl: CD® (SD) BR	Benign neoplasm in adrenal gland, medulla	No	Possible	No data	Single (M)	No	Oral	No information retrievable
CD®-1 albino mice	None	No	No	Not applicable	Not applicable	No (body weight gains at 18 months were decreased by 6 % in males at 800 ppm (not statistically significant)	Oral	-

10.9.2 Comparison with the CLP criteria

There are no relevant data from epidemiological studies; hence, classification with Cat 1A according to CLP regulation is not justified.

The increase in benign medullary neoplasms of adrenals in male rats is not regarded as sufficient evidence for carcinogenicity in animals. Accordingly, Cat. 1B is not required. Classification into Cat 2 is not required for the following reasons, according to Guidance of the Application of the CLP criteria 3.6.2.3.2: Incidences for benign adrenal neoplasms were numerically increased, but that increase did not reach statistical significance neither in pairwise comparisons nor in trend testing. Findings were observed in males only. In addition, there were no effects in the adrenal gland in the subchronic studies thus showing that the adrenal gland seems not to be a specific target organ or concern.

10.9.3 Conclusion on classification and labelling for carcinogenicity

In summary, no classification for carcinogenic effects is proposed by DS.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No information on the carcinogenicity of picolinafen in humans is available. The carcinogenicity of picolinafen has been investigated in one 24-month study in rats and in one 18-month study in mice by the oral route. There were no carcinogenicity studies in animals by inhalation or dermal route. The results are summarised in the table below.

Table: *Summary table of animal studies on carcinogenicity*

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>24-month study in rats

OECD TG 453

GLP

Supplementary (survival at 24 months: 24 - 31 % for males, 33% - 43% for females)

Sprague Dawley rats Crl: CD® (SD) BR

65 M + 65 F per group</td><td>Picolinafen technical (Batch CA14113; 97.8% as)

2.4/3.0, 12.1/15.0, 24.5/31.0 mg/kg bw/d for m/f

24 months</td><td>Neoplastic effects: At the top dose, increased incidence of benign and malignant neoplasms in the adrenal gland (medulla) in males
<table>
<tr><th>Dose group (ppm)</th><th>0</th><th>50</th><th>250</th><th>500</th></tr>
<tr><td colspan="5">12-mo interim sacrifice</td></tr>
<tr><td>Animals examined</td><td>10</td><td>0</td><td>1</td><td>10</td></tr>
<tr><td>Medulla: benign neoplasm (unilateral)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Medulla: benign neoplasm (bilateral)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Medulla: malignant neoplasm (unilateral)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="5">Unscheduled Deaths</td></tr>
<tr><td>Animals examined</td><td>42</td><td>40</td><td>39</td><td>40</td></tr>
<tr><td>Medulla: benign neoplasm (unilateral)</td><td>3</td><td>5</td><td>2</td><td>4</td></tr>
<tr><td>Medulla: benign neoplasm (bilateral)</td><td>1</td><td>1</td><td>0</td><td>0</td></tr>
<tr><td>Medulla: malignant neoplasm (unilateral)</td><td>0</td><td>1</td><td>1</td><td>1*</td></tr>
<tr><td colspan="5">Terminal sacrifice</td></tr>
<tr><td>Animals examined</td><td>13</td><td>2</td><td>3</td><td>15</td></tr>
<tr><td>Medulla: benign neoplasm (unilateral)</td><td>2</td><td>0</td><td>2</td><td>4</td></tr>
<tr><td>Medulla: benign neoplasm (bilateral)</td><td>0</td><td>1</td><td>0</td><td>3</td></tr>
<tr><td>Medulla: malignant neoplasm</td><td>0</td><td>1</td><td>1</td><td>1</td></tr>
</table></td><td>Anonymous 18, 1999</td></tr>
</table>

<table>
<tr><td></td><td></td><td>
<table>
<tr><td>(unilateral)</td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="5">All animals</td></tr>
<tr><td>Animals examined</td><td>65</td><td>42</td><td>43</td><td>65</td></tr>
<tr><td>Medulla: benign neoplasm (unilateral)</td><td>5 (7.7%)</td><td>5 (11.9%)</td><td>5 (11.6%)</td><td>8 (12.3%)</td></tr>
<tr><td>Medulla: benign neoplasm (bilateral)</td><td>1 (1.5%)</td><td>2 (4.8%)</td><td>0 (0.0%)</td><td>3 (4.6%)</td></tr>
<tr><td>Medulla: malignant neoplasm (unilateral)</td><td>0 (0.0%)</td><td>2 (4.8%)</td><td>2 (4.7%)</td><td>1 (1.5%)</td></tr>
</table>
*Metastasis from lympho-reticular system

The range of frequencies of unilateral benign neoplasms in the adrenal gland (medulla) in control groups of 5 studies (HCD) performed between 1991 and 1995 was from 0 to 9.8% (0%, 0%, 9.8%, 0%, 5% and frequencies of bilateral benign neoplasms was from 0 to 3.9% (0%, 0%, 3.9%, 0% and 1.6%). No HCD for unilateral malignant neoplasm in medulla was provided.
Survival rates were for control group, 50, 250 and 500 ppm groups, respectively: 24%, 29%, 31% and 29% for males and 42%, 43%, 33% and 35% for females.</td><td></td></tr>
<tr><td>18-months study in mice

OECD TG 451

GLP

Acceptable

CD®-1 albino mice

65 M + 65 F per group</td><td>Picolinafen technical (Batch CA14113; 97.8% as)

0, 6.9/8.2, 68.6/81.0, 137.1/165.8 mg/kg bw/d for m/f

18 months</td><td>No treatment-related increase in the type or incidence of tumours

Haematology (increased reticulocyte counts and MCHC); liver (increased weights, hypertrophy); spleen pigment</td><td>Anonymous 17, 1999</td></tr>
</table>

The DS considered that classification into Carc. 2 is not required. The incidence for benign adrenal neoplasms was numerically slightly increased, but that increase did not reach statistical significance neither in pairwise comparisons nor in trend test. Findings were observed in male rats only. In addition, there were no effects in the adrenal gland in the subchronic studies thus showing that the adrenal gland seems not to be a specific target organ or concern.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The carcinogenicity of picolinafen was examined in the 24-month study in rats and in the 18-month study in mice. No increase in neoplasm frequency was observed in the study in mice. In the study in rats, only a slight increase in the incidence of benign neoplasms in the adrenal gland (medulla) was observed in the male, but not in the female rats. This slight increase was not statistically significant in comparison with the concurrent control group. Since no dose-response relationship was observed and the incidence of these tumours in the concurrent control group was close to the upper border of the incidence range for uni/bilateral benign neoplasms in medulla in five historical control studies, this finding is not an evidence of carcinogenicity of picolinafen. Lack of mutagenicity of picolinafen lowers the concern and supports the opinion that this small, not statistically significant increase in incidence of benign neoplasms was likely occurring by chance. Taking that into account, RAC concurs with the DS opinion that picolinafen does not warrant classification for carcinogenicity.

10.10 Reproductive toxicity

Results of the available reproductive toxicity and developmental toxicity studies are summarised in Table 26 and Table 28.

10.10.1 Adverse effects on sexual function and fertility

Table 26: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
2-generation study OECD TG 416 GLP Sprague Dawley rats 30 M, 30 F	Picolinafen technical (Batch CA14113; 97.8 % as) 0, 50, 250 or 500 ppm Corresponding to: 0, 3.7/4.2, 18.8/22.1, 38.7/44.1 mg/kg bw/d for m/f in pre mating period P and F1 generation: treatment for 10 weeks prior to a 14-day mating period; treatment of parental generation continued curing the mating period and post-	statistically significant reduction in testicular sperm counts in P generation (18.8 mg/kg bw/d: 85.4 million sperm/g of tissue (-16.6 %) and at 38.7 mg/kg bw/d 88.1 million sperm/g of tissue (-14 %) compared to 102.4 in control group) 38.7 mg/kg bw/d: statistically significant reduction in epididymal sperm count in P generation (546.2 million sperm/gram of tissue compared to 757.8 million sperm/gram of tissue in control group, -27.9 %) Other effects: weight changes in liver and kidney and evidence of anemia at 19/22 mg/kg bw/d for m/f and 39/44 mg/kg bw/d for m/f	Anonymous 21, 1999

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	mating period		

In the 2-generation reproduction study conducted with Sprague-Dawley rats (Anonymous 21, 1999), anaemia was noted for both parental generations, as evidenced by changes in haematological parameters, increased absolute and relative spleen weights, and microscopic changes in the spleen. Anaemia was also noted for F2 pups on postnatal Day 21, as evidenced by changes in haematological parameters (haematology examinations were only conducted for F2 pups on postnatal Day 21).

At 18.8 and at 38.7 mg/kg bw/d, testicular sperm count was statistically significant reduced in P-generation by 16.6 % and 14 % (85.4 and 88.1 million sperm/gram of tissue, respectively compared to 102.4 million sperm/gram of tissue in control group). In F1 generation, no decrease in testicular sperm count was seen. The applicant submitted HCD from 9 studies, in which range of sperms per grams is between 82.1 and 141.6 million sperm/gram of tissue. However, no further information about quality of HCD is given and hence, HCD are not reliable.

At 38.7 mg/kg bw/d, epididymal sperm count was statistically significant reduced in P-generation by 27.9 % (546.2 million sperm/g of tissue compared to 757.8 million sperm/g of tissue in control group). The applicant cited HCD, however, as no further information about quality of HCD is given, study internal control data should be used.

In the 90 day rat study (Anonymous 10, 1998), absolute and relative testes weight were not affected. One of 10 animals at highest dose level (65.4 mg/kg bw/d) showed unilateral diffuse atrophy of testes, and 1 of only 1 examined animal at the next lower dose level (32.2 mg/kg bw/d), whereas none animal in control group). 1 of 10 animals showed unilateral hypospermia in epididymis at 65.4 mg/kg bw/d.

In the 90-day dog study (Anonymous 12, 1999), absolute and relative testes weight were not affected. Histopathology of testes and epididymis was inconspicuous.

In the 1-year dog study (Anonymous 13, 1999), absolute and relative testes weight were not affected. Degeneration/atrophy of germinal epithelium in testes was seen without dose response (see following table).

	0 mg/kg bw/d	**1.4 mg/kg bw/d**	**4.4 mg/kg bw/d**	**42.7 mg/kg bw/d**
testes	**N=4**	**N=4**	**N=4**	**N=4**
Unilateral germinal epithelium: degeneration/atrophy	1 (minimal)	3 (1 minimal, 2 slight)	0	2 (minimal)
Bilateral germinal epithelium: degeneration/atrophy	2 (slight)	0	0	1 (minimal)

Picolinafen technical did not affect reproductive performance.

10.10.2 Comparison with the CLP criteria

Table 27 presents the toxicological results in comparison with CLP criteria.

Table 27: Toxicological results concerning adverse effects on sexual function and fertility

Toxicological result	**CLP criteria**
2-generation reproduction study in rats, Picolinafen administered via diet: No effects on fertility or reproduction observed up to highest dose tested (500 ppm, 39/44 mg/kg bw/d)	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and - where the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

There are no epidemiological data to evaluate effects on fertility, hence Picolinafen cannot be placed in category 1A (CLP). In the submitted multigeneration study in rats, no findings with relevance for a classification for adverse effects on sexual function and fertility were reported. It could be discussed whether the statistical significant reduction in testicular sperm counts in P-generation at the two highest dose level and the statistical significant reduction in epididymal sperm count in P-generation in the 2 generation study in rats are sufficient to justify classification. However, as no significant effects on weight or histopathology of testes/epididymides were seen in 90-day rat, 90-day dog or 1-year dog study, DS decided not to propose classification for these effects.

10.10.3 Adverse effects on development

Developmental toxicity studies were performed in rabbits and in rats. Results are summarized in Table 28.

Table 28: Summary table of animal studies on adverse effects on development

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Oral
teratology
OECD TG 414
GLP
New Zealand White rabbits
25 F</td><td>Picolinafen technical (Batch CA14113; 97.8 % as)
5, 20, 50 mg/kg bw/d
From days 6-28 of gestation</td><td>No increased incidence in malformations
At 50 mg/kg bw/d: statistically significant increased incidence in fused sternal centra (but: not considered treatment-related, because the litter incidence was not statistically significant increased, fetal incidence was within the range of HCD)
<table>
<tr><td colspan="2">Dosage group (mg/kg bw/d)</td><td>0</td><td>5</td><td>20</td><td>50</td></tr>
<tr><td colspan="2">Litters evaluated</td><td>23</td><td>24</td><td>22</td><td>20</td></tr>
<tr><td colspan="2">Fetuses evaluated</td><td>190</td><td>226</td><td>192</td><td>161</td></tr>
<tr><td rowspan="2">Sternal centra: fused</td><td>Litter incidence</td><td>2</td><td>0</td><td>1</td><td>3</td></tr>
<tr><td>Fetal incidence</td><td>3</td><td>0</td><td>1</td><td>6*</td></tr>
</table>
Other effects:
<table>
<tr><td>Dosage group (mg/kg bw/d)</td><td>0</td><td>5</td><td>20</td><td>50</td></tr>
<tr><td>Rabbits tested</td><td>25</td><td>25</td><td>25</td><td>25</td></tr>
<tr><td>Pregnant</td><td>24</td><td>25</td><td>23</td><td>25</td></tr>
<tr><td>Found dead</td><td>0</td><td>1</td><td>0</td><td>1</td></tr>
<tr><td>Aborted</td><td>1</td><td>0</td><td>1</td><td>2</td></tr>
<tr><td>Prematurely delivered</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Resorptions, early</td><td>5</td><td>3</td><td>8</td><td>11</td></tr>
<tr><td>Resorptions, late</td><td>3</td><td>4</td><td>2</td><td>7</td></tr>
<tr><td>Does with any resorptions</td><td>5</td><td>6</td><td>7</td><td>7</td></tr>
<tr><td>Mean life fetal body weight (g/litter)</td><td>43.06</td><td>40.74</td><td>37.90**</td><td>40.13</td></tr>
</table>
Group mean maternal hematology
<table>
<tr><td>Dosage group (mg/kg bw/d)</td><td>0</td><td>5</td><td>20</td><td>50</td></tr>
<tr><td>HB (g/dl)</td><td>12.37</td><td>12.16</td><td>11.53</td><td>10.71</td></tr>
<tr><td>HCT (%)</td><td>36.84</td><td>35.88</td><td>34.00</td><td>31.36</td></tr>
<tr><td>RBC (10^6/µL)</td><td>5.612</td><td>5.455</td><td>4.998**</td><td>4.107**</td></tr>
<tr><td>RETIC (% RBC)</td><td>1.13</td><td>1.28</td><td>2.24**</td><td>2.81**</td></tr>
</table>
HB = haemoglobin concentration; RBC = erythrocyte count; HCT = hematocrit; RETIC = reticulocyte Count
* ($p \leq 0.05$) significantly different from control (Dunnett`s test)<br>** ($p \leq 0.01$) significantly different from control (Dunnett`s test)</td><td>Anonymous 19, 1998</td></tr>
<tr><td>Oral</td><td>Picolinafen</td><td>No adverse effects in foetuses or in development reported</td><td>Anonymous</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Teratology Toxicity
OECD TG 414
GLP
Sprague Dawley rats
25 F</td><td>technical (Batch CA14113; 97.8 % as)
0, 5, 25, 50, 100, 500, 1000 mg/kg bw/d
Day 6 – 19 of gestation</td><td>Other effects:
Group Mean Maternal Hematology:
<table>
<tr><td>Dosage group (mg/kg bw/d)</td><td>0</td><td>100</td><td>500</td><td>1000</td></tr>
<tr><td>HB (g/dl)</td><td>12.00</td><td>11.20**</td><td>11.17**</td><td>11.11**</td></tr>
<tr><td>HCT (%)</td><td>32.44</td><td>30.14**</td><td>29.78**</td><td>29.33**</td></tr>
<tr><td>RBC (10^6/µL)</td><td>5.10</td><td>4.59**</td><td>4.18**</td><td>3.98**</td></tr>
<tr><td>RETIC (10^3/µL)</td><td>2.06</td><td>2.65*</td><td>3.75**</td><td>4.42**</td></tr>
<tr><td>Dosage group (mg/kg bw/d)</td><td>0</td><td>5</td><td>25</td><td>50</td></tr>
<tr><td>HB (g/dl)</td><td>11.51</td><td>11.81</td><td>11.32</td><td>11.25</td></tr>
<tr><td>HCT (%)</td><td>32.39</td><td>33.42</td><td>32.01</td><td>32.14</td></tr>
<tr><td>RBC (10^6/µL)</td><td>4.966</td><td>5.075</td><td>4.880</td><td>4.947</td></tr>
<tr><td>RETIC (% RBC)</td><td>1.28</td><td>1.36</td><td>1.31</td><td>1.50</td></tr>
</table>
HB = haemoglobin concentration; RBC = erythrocyte count; HCT = hematocrit; RETIC = reticulocyte Count
* ($p \leq 0.05$) significantly different from control (Dunnett`s test)
** ($p \leq 0.01$) significantly different from control (Dunnett`s test)

Mean Terminal Body Weights and Spleen Weights Data
<table>
<tr><td>Dosage group (mg/kg bw/d)</td><td>0</td><td>100</td><td>500</td><td>1000</td></tr>
<tr><td>Rats tested</td><td>25</td><td>25</td><td>25</td><td>25</td></tr>
<tr><td>Terminal body weight (g)</td><td>384.5</td><td>378.4</td><td>368.2*</td><td>362.7**</td></tr>
<tr><td>Absolute spleen weight (g)</td><td>0.67</td><td>0.76*</td><td>0.90**</td><td>0.88**</td></tr>
<tr><td>Spleen/terminal body weight ratio (%)</td><td>0.173</td><td>0.202**</td><td>0.247**</td><td>0.244**</td></tr>
</table>
* ($p \leq 0.05$) significantly different from control (Dunnett`s test)
** ($p \leq 0.01$) significantly different from control (Dunnett`s test)

Maternal absolute feed consumption values (g/day)
<table>
<tr><td>Dosage group (mg/kg bw/d)</td><td>0</td><td>100</td><td>500</td><td>1000</td></tr>
<tr><td>Rats tested</td><td>25</td><td>25</td><td>25</td><td>25</td></tr>
<tr><td>Maternal feed consumption (g/day) Days 6-20</td><td>79.2</td><td>78.3</td><td>74.6**</td><td>72.8**</td></tr>
</table>
**($p \leq 0.01$) significantly different from control</td><td>20, 1999</td></tr>
</table>

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

The prenatal developmental toxicity was investigated in rats (Anonymous 19, 1998) and rabbits (Anonymous 20, 1999) complying with international test guidelines and GLP. Developmental toxicity tests conducted with Picolinafen technical in Sprague-Dawley rats and New Zealand White rabbits revealed no evidence of teratogenic effects in either the rat or rabbit. In the rat, maternal toxicity was evidenced by reductions in food consumption, mean body weights and body weight gains, as well as haematological changes, increased absolute and relative spleen weights and microscopic splenic changes indicative of anaemia.

In the rabbit, maternal toxicity was evidenced by reductions in food consumption and body weight gains, as well as haematological changes and microscopic splenic changes indicative of anaemia. An increase in resorption rate and decreased mean foetal body weights were also noted in this study.

10.10.5 Comparison with the CLP criteria

Table 29 presents the results of the valid toxicological studies in comparison with the CLP criteria for reproductive toxicity.

Table 29: Toxicological results concerning adverse effects on development

Toxicological result	**CLP criteria**
Teratology study in rats: No effects on foetuses observed up to highest dose tested (1000 mg/kg bw/d)	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on development in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and - the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects
Teratogenicity study in rabbits: No increased incidences of malformations reported up to highest dose tested (50 mg/kg bw/d) Increased rate of late resorptions and lower mean foetal body weight at 50 mg/kg bw/d. Increased rate of abortions at 50 mg/kg bw/d. At 20 mg/kg bw/d and above: lower feed intake and body weights, indications of anaemia (haematological changes, histological findings in spleen)	

There are no appropriate epidemiological studies available on developmental effects in humans. Hence, classification with Category 1A according CLP regulation is not possible.

In rats, no findings in offspring relevant for a possible classification for developmental effects were reported.

In rabbits, no increased rates of malformations were reported. Slight increases in late resorptions and in abortions were observed in the top dose group of 50 mg/kg bw/d. These slight increases are considered not sufficiently severe to trigger classification for developmental effects. From 20 mg/kg bw/d onwards, signs of

maternal toxicity (lower feed intake and body weights as well as haematological changes being indicative of anemia) were seen.

In summary, neither classification in Category 1B (H360D) nor Category 2 (H361d) according to CLP criteria is considered appropriate.

10.10.6 Adverse effects on or via lactation

No data are available to judge whether there are specific effects on or via lactation (H362).

10.10.7 Conclusion on classification and labelling for reproductive toxicity

Based on the available evidence, Picolinafen should not be classified for reproductive toxicity.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The reproductive toxicity of picolinafen has been studied in rats and rabbits. For effects on sexual function and fertility, the DS presented the results of one two-generation study in rats, supplemented with data on effects in sex organs from the repeated-dose toxicity studies in rats and dogs. For developmental toxicity, one developmental toxicity study (oral) in rats and one developmental toxicity study (oral) in rabbits were presented.

Based on results of these studies the DS concluded that picolinafen should not be classified for reproductive toxicity.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

The effect of picolinafen on fertility and sexual function was assessed based on the results from the 2-generation study in rats and the repeated dose toxicity studies in rats and dogs.

<u>2-generation study</u>

In the 2-generation study in rats (30/sex/dose) performed according to OECD TG 416 (Anonymous 21, 1999), picolinafen was given to the P-generation in diet at concentrations of 0, 50, 250 or 500 ppm for 10 weeks prior to a 14-day mating period, during mating, gestation, and lactation periods. After weaning of the F1-generation, at 4 weeks of age, selected weanlings were maintained in the same dietary groups through maturation, mating, gestation, and lactation. The achieved doses in mg/kg bw/d in the

50, 250 or 500 ppm groups of rats, as calculated during the pre-mating phase, were: 0, 3.7/4.2, 19/22 and 39/44 mg/kg bw/d for males/females.

Parental toxicity: Anaemia was noted for both parental generations, as evidenced by changes in haematological parameters, increased absolute and relative spleen weights, and microscopic changes in the spleen. Anaemia was also noted for F2-pups on postnatal day 21.

Fertility and sexual functions: No effects on fertility and sexual behaviour were reported.

Sperm measurements: In P-generation males, exposed at mid dose of 19 mg/kg bw/d and at top dose of 39 mg/kg bw/d, sperm count in testes was statistically significantly reduced by 16.6% and 14% (85.4 and 88.1 million sperm/gram of tissue, respectively compared to 102.4 million sperm/gram of tissue in control group). However, in F1-generation males exposed at the same dose levels no decrease in testicular sperm count was seen. The applicant submitted historical control data (HCD) from 9 studies, in which range of sperms per grams is between 82.1 and 141.6 million sperm/gram of testes tissue. However, no further information about quality or relevance of HCD was given.

In P-generation males exposed at the top dose of 39 mg/kg bw/d sperm count in epididymis was statistically significantly reduced by 27.9% (546.2 million sperm/g of tissue compared to 757.8 million sperm/g of tissue in control group). The quality of the HCD were low and not suitable for comparison. In F1-generation no reduction of epidydimal sperm count was observed in any of the groups of males exposed to picolinafen.

The observation of lower sperm counts in male rats of P-generation at the two highest dose levels indicate that the testes might be a target organ, but no dose response relationship was observed and the sperm counts in these animals were within HCD, although the relevance of HCD was not clearly demonstrated. In addition, this effect on sperm count was observed only in males in P-generation but not in F1-generation although exposure levels were similar, and the sperm counts in affected males were relatively high. This effect is not considered as sufficient to demonstrate evidence for classification of picolinafen to Repr. 2. This conclusion is further supported by results in the repeated dose toxicity studies in rats and dogs (see below).

<u>Repeated-dose toxicity studies</u>

In the 90-day rat study (Anonymous 10, 1998), absolute and relative testes weight were not affected. Unilateral diffuse atrophy of testes was seen in one out of 10 animals at highest dose level (65.4 mg/kg bw/d), and in 1 of only 1 examined animal at the next lower dose level (32.2 mg/kg bw/d), whereas no male in the control group showed this effect. One out of 10 animals showed unilateral hypospermia in epididymis at 65.4 mg/kg bw/d.

In the 90-day dog study (Anonymous 12, 1999), absolute and relative testes weight were not affected. Histopathology of testes and epididymis was inconspicuous.

In the 1-year dog study (Anonymous 13, 1999), absolute and relative testes weight were not affected. Degeneration/atrophy of germinal epithelium in testes was seen without dose response (see following table).

***Table**: 1-year dog study: information on testes*

	0 mg/kg bw/d	**1.4 mg/kg bw/d**	**4.4 mg/kg bw/d**	**42.7 mg/kg bw/**
testes	**N=4**	**N=4**	**N=4**	**N=4**
Unilateral germinal epithelium: degeneration/atrophy	1 (minimal)	3 (1 minimal, 2 slight)	0	2 (minimal)
Bilateral germinal epithelium: degeneration/atrophy	2 (slight)	0	0	1 (minimal)

Taking into account the lack of effect on fertility and sexual behaviour in the 2-generation study in rats and lack of clear-cut effect on testes in rats in the 90-day repeated toxicity study and in the 90-day and 1-year repeated toxicity studies in dogs, RAC is of the opinion that picolinafen does not warrant classification for fertility and sexual function concurring with the opinion of the DS.

Developmental toxicity

There were two prenatal toxicity studies performed in accordance with the OECD TG 414 and in GLP conditions, one in Sprague-Dawley rats (Anonymous 20, 1999) and one in New Zealand White rabbits (Anonymous 19, 1998).

In rats and in rabbits, maternal toxicity was evidenced by reductions in food consumption, and body weight gains, as well as haematological changes, increased spleen weights and microscopic splenic changes indicative of anaemia.

In the prenatal toxicity study in rabbits (Anonymous 19, 1998), abortions were not significantly increased (1 out of 25 does in the control group, 1 out of 25 does in the 20 mg/kg bw/d group and in 2 out of 24 does the 50 mg/kg bw/d group had abortions). One doe in the 50 mg/kg bw/d group prematurely delivered on gestation day 29. At 50 mg/kg bw/d the clinical signs of maternal toxicity, in addition to those mentioned above, included also a slightly increased incidence of soft or liquid faeces for does compared to controls. Slight, not statistically significant increases in the total number of resorptions and the mean resorption rate were noted at 50 mg/kg bw/d compared to controls. In the control group, a total of 8 resorptions (5 early and 3 late resorptions) were noted in 5 out of 23 litters, while a total of 18 resorptions (11 early and 7 late resorptions) in 7 out of 20 litters were noted at 50 mg/kg bw/d. The mean resorption rate (the total number of resorptions divided by the total number of litters) was 0.3 (8 resorptions/23 litters) for controls versus 0.9 (18 resorptions/21 litters) for the 50 mg/kg bw/d group. The incidence of abortions and incidence of resorptions in the 50 mg/kg bw/d group with moderate maternal toxicity was only slightly, not significantly higher than in the control group providing only limited evidence for developmental toxicity.

Since the rat and rabbit studies revealed no evidence of teratogenic or other treatment-

related developmental effects meeting classification criteria, RAC is of the opinion that picolinafen does not warrant classification for developmental toxicity.

Effects on or via lactation

Since no data were provided to judge whether there are specific effects on or via lactation (H362), picolinafen should not be classified for this hazard category due to lack of data.

10.11 Specific target organ toxicity-single exposure

In two acute toxicity studies, signs of clinical toxicity were observed. The studies are summarised in the following table.

Table 30: Summary table of animal studies relevant to classification for STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Acute Dermal Toxicity OECD TG 402 GLP Sprague Dawley rat 5 M, 5 F	Picolinafen technical (Batch CA14113; 97.8 % as) 4000 mg/kg bw, 24 hours exposure period	4000 mg/kg bw: body weight loss in one female	Anonymous 10, 1998
Acute Inhalation Toxicity (nose-only) OECD TG 403 GLP Sprague Dawley rat 5 M, 5 F	Picolinafen technical (Batch CA14113; 97.8 % as) administered as a dust (milled prior to administration) MMAD: 5.8 microns with a geometric standard deviation of 1.6 microns 4 hours via nose-only inhalation	5.9 mg/L: labored breathing, moist rales, clear nasal discharge, salivation, chromodacryorrhea	Anonymous 7, 1997

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

Clinical signs of toxicity were observed in two studies. In the acute dermal toxicity study (Anonymous 10, 1998), body weight loss (4 g) was observed in one female rat at 4000 mg/kg bw. In the acute inhalation study (Anonymous 7, 1997), several symptoms (labored breathing, moist rales, clear nasal discharge, salivation, chromodacryorrhea) were seen during the first two hours following exposure to the substance. These findings continued during study days 2 and 3, but were resolved by study day 4.

10.11.2 Comparison with the CLP criteria

Table 31 presents the results of the dermal toxicity and the inhalation study with the CLP criteria for STOT SE.

Table 31: Classification criteria for Categories 1 and 2 of specific target organ toxicity-single exposure (C: guidance value)

Toxicological data	**CLP criteria**	
Clinical signs of toxicity were noted in the acute dermal toxicity study in rats at 4000 mg/kg bw (body weight loss) and in acute inhalation study in rats at 5.9 mg/L, 4 hours exposure (labored breathing, moist rales, clear nasal discharge, salivation, chromodacryorrhea).	Category 1 (H370) Oral (rat): C ≤ 300 mg/kg bw Dermal (rat or rabbit): C ≤ 1000 mg/kg bw Inhalative (rat, dust/mist/fume): ≤ 1 mg/L/4 h	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure - reliable and good quality evidence from human cases or epidemiological studies; or - observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations.
	Category 2 (H371) Oral (rat): 2000 ≥ C > 300 mg/kg bw Dermal (rat or rabbit): 2000 ≥ C > 1000 mg/kg bw Inhalative (rat, dust/mist/fume): 5 ≥ C > 1 mg/L/4 h	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure - observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.
	Category 3 (H335/H336) Guidance values do not apply (mainly based on human data)	Transient target organ effects This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.

10.11.3 Conclusion on classification and labelling for STOT SE

The observed non-lethal effects reported after acute exposure occurred above the respective guidance values, were transient and were not of considerably adverse nature with no significant impact on health. Hence, no classification with STOT SE is proposed.

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

Summary of the Dossier Submitter's proposal

The DS noted that the observed non-lethal effects reported after acute dermal and inhalation exposure occurred above the respective guidance values. These effects were transient and were not of considerably adverse nature with no significant impact on health. Hence, no classification with STOT SE was proposed.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

There are no data in humans on specific target organ toxicity single exposure of picolinafen.

In the acute dermal toxicity study (Anonymous 10, 1998), body weight loss (4 g) was observed only in one female rat at 4000 mg/kg bw.

In the acute inhalation study laboured breathing was noted during the 4-hour exposure period. Also, moist rales, clear nasal discharge, salivation and red lacrimal secretion were observed in rats exposed to dust of picolinafen at one very high, although non-lethal, concentration of 5.9 mg/L (5.9 g/m^3) during the first 2 hours following exposure (Anonymous 7, 1997). These responses continued during the first two days following exposure (study days 2 and 3) and were resolved for all animals by study day 4. All animals gained weight during the 14-day post-exposure observation period, and no macroscopic findings were noted at necropsy. No histopathological examinations of upper respiratory tract were done, so histopathological changes cannot be evaluated.

The existing data are considered by RAC as not conclusive for classification to STOT SE category, therefore no classification is warranted.

10.12 Specific target organ toxicity-repeated exposure

Table 32: Summary table of animal studies on studies relevant to classification for STOT RE

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

28 day oral study in rats Comparable to OECD TG 407 GLP Sprague Dawley rats, Crl:CD (SD)BR 10 M, 10 F	Picolinafen technical (Batch No. 4; 100.2 % as) Fed at dietary concentrations of 0, 2.7/3.0, 5.4/5.9, 10.5/11.7, 107/119 mg/kg bw/d for m/f for 28 days	At 107 mg/kg bw/d: statistically significant haematological changes indicative of regenerative hemolytic anaemia Reduction of HB by 9.1 % in males, by 12.3 % in females Reduction of HCT by 9.8 % in females Reduction in RBC by 11.4 % in males, by 18 % in females Increase in reticulocytes by 111 % in males, by 83 % in females Methaemoglobin formation (1.8 % compared to 0% in control group in males, 1.23 % in compared to 0.01% in females) Formation of Heinz bodies (5.6 % compared to 0 % in control group in males, 6.8 % compared to 0 % in males) statistically significant increase in plasma bilirubin in both sexes (by 48 % in males and by 108 % in females) **Spleen:** Statistical significant increase in relative spleen weight (by 83 % in males, by 79 % in females), 10 of 10 spleens enlarged and discoloured (0 in control group) in males and females, extramedullary hematopoiesis in all 10 males (9 moderate, 1 slight) and all 10 females (1 severe, 9 moderate), hemosiderin deposition in all 10 males (7 severe, 3 moderate) and all 10 females (9 severe, 1 moderate), focal capsular inflammation /capsular fibrotic proliferation (in 4 males compared to 1 in control group; in 4 females compared to 0 in control group) **Liver:** Statistical significant increase in relative liver weight (by 8 % in males and females), erythropoietic foci in all 10 males, in 6 of 10 females, centrilobular hypertrophy in 9 of 10 males (0 in control group), Kupffer cell hemosiderin in all males (7 slight, 3 very slight compared to 0 in control group) and in all females (1 moderate, 9 slight) **Kidney:** Intra-epithelial tubular haemosiderin in kidney in 3 of 10 males and 7 of 10 females Statistical significant increase in creatinine in females **Bone marrow** of femur/joint: increased erythropoiesis in 9 of 10 females	Anonymous 15, 1993

28 day oral study in mice OECD TG 407 GLP CD-1 albino mice 5 M, 5 F	Picolinafen technical (Batch CP29327; 99.5 % as) Fed at dietary concentrations of 0, 23.4/28.0, 227.2/234.9, 437.8/597.7, 863.9/1140.3, 1721.1/2019.4 mg/kg bw/d for m/f for 28 days	At 227.2 mg/kg bw/d and above: findings indicative of anaemia (extramedullary hematopoiesis in the spleen, brown pigmentation of spleen) **Clinical signs** being indicative of anaemia: pale extremities in 5 of 5 male and 3 of 5 female animals at 864 mg/kg bw/d and 5 of 5 male and 5 of 5 female animals at 1721 mg/kg bw/d **Macroscopic findings**: paleness of kidney, liver, spleen, lung, heart **Haematological changes** being indicative of anaemia: Decrease in red blood cells in females at 2019. 4 mg/kg bw/d (5.79 x 10^6/µl compared to 6.87 x 10^6/µl in control group) Increase in reticulocytes in both sexes at 864 mg/kg bw/d (females 4.78 x 10^3/µl compared to 1.36 x 10^3/µl in control group, males 4.3 x 10^3/µl compared to 1.48 x 10^3/µl in control group) and at 1721 mg/kg bw/d (females 6.56 x 10^3/µl compared to1.36 x 10^3/µl in control group, males 10.64 x 10^3/µl compared to 1.48 x 10^3/µl in control group) increase in MCV in males at 1721 mg/kg bw/d (54.7 fl compared to 47.4 fl in control group) and females (statistically significant at 2019 mg/kg bw/d: 53.5 fl compared to 48.6 fl in control group) increase in MCH at 864 mg/kg bw/d (males statistically significant 21.1 pg compared to 17.6 pg in control group, females 21.5 pg compared to 18.2 pg in control group) and at 1721 mg/kg bw/d (males 21.8 % compared to 17.6 pg in control group, females 24.6 pg compared to 18.2 pg in control group) and MCHC in both sexes from 864 mg/kg bw/d onwards (males 42.8 % at 863 mg/kg bw/d, 39.9 % at 1721 mg/kg bw/d compared to 37.2 % in control group, females 42.8 % at 863 mg/kg bw/d, 45.9 % at 1721 mg/kg bw/d compared to 37.3 % in control group) increase in Heinz body formation in females at 235 mg/kg bw/d (1x10^3/µl), at 598 mg/kg bw/d (1.2 x10^3/µl), at 864 mg/kg bw/d (statistically significant, 2.8 x 10^3/µl) and in both sexes at 1721 mg/kg bw/d (statistically significant, males: 3x10^3/µl compared to 0.4 x 10^3/µl in control group, females: 4 x10^3/µl compared to 0.2 x 10^3/µl in control group) **Spleen**: brown pigment deposition in all males and females from 227 mg/kg bw/d onwards, extramedullary haematopoiesis (4 of 5 males and 4 of 5 females at 227 mg/kg bw/d, all animals at higher dose levels compared to 0 in control group), increase in absolute weight in both sexes from 438 mg/kg bw/d onwards **Liver**: pigment deposition in Kupffer cells (statistically significant in both sexes, 5 of 5 males and 4 of 5 females at 438 mg/kg bw/d and all animals at higher dose levels), increase in AST, ALT, increase in absolute weight from 438 mg/kg bw/d onwards in both sexes Single cell necrosis in one female at 1140 mg/kg bw/d, in 3 males and 2 females at 1721 mg/kg bw/d	Anonymous 14, 1998

28 day oral study in dogs No TG available GLP Beagle dogs 2 M, 2 F	Picolinafen technical (Batch CA14113; 97.8 % as) Fed at dietary concentrations of 0, 3.9/5.1, 47.7/43.9, 90.4/71.5, 313.4/248.5 mg/kg bw/d for m/f for 28 days	At 43.9 mg/kg bw/d: Thyroid/parathyroids (increased weights, enlarged, hyperplasia and hypertrophy of follicular epithel cells); elevated serum cholesterol levels At 71.5 mg/kg bw/d: haematological changes being indicative of haemolytic anemia: increased reticulocyte count (in one of two females, 4.2 % compared to 2.7 % in pre-test) Decreased haemoglobin (-19 % in males, - 16 % in females compared to control group) decreased haematocrit (-8 % in males, -16 % in females compared to control group) At 248.5 mg/kg bw/d: Decreased haemoglobin (-27 % in males, - 28 % in females compared to control group) Decreased haematocrit (-21.5 % in males, -27 % in females compared to control group) Decreased red blood cell counts (-28 % in males, -30 % in females compared to control group) Increased reticulocyte counts (+69 % in males, +385 % in females) Liver: enlarged liver in one male at 43.9 mg/kg bw/d, in all animals at higher dose levels	Anonymous 11, 1998

90 day oral study in rats OECD TG 408 GLP Sprague Dawley derived rats	Picolinafen technical (Batch CP29327; 99.5 % as) Fed at dietary concentrations of 0, 6.4/6.8, 32.2/35.1, 65.4/69.0 mg/kg bw/d for m/f for 13 weeks	At 32.2 mg/kg bw/d: Haematological and histopathological changes being indicative of haemolytic anemia: 32.2 mg/kg bw/d: statistically significant decreases in HGB (at day 57: -9 % in males, -10 % in females, at day 92/day 93: -12 % in males, -12 % in females) statistically significant decreases in HTC (day 29: – 12 % in males, day 57: - 7 % in males, day 92 - 8 % in males, day 93: – 5 % in females) statistically significant decreases in RBC (day 29: -15 % in males, day 57: -10 % in males, day 92: -11 % in males, day 93: -7 % in females) Pigment deposition, hemosiderin in spleen (9 of 10 male rats, 10 of 10 female rats) Pigment deposition in Kupffer cells in liver (7 of 10 male rats, 8 of 10 female rats) 65.4 mg/kg bw/d: statistically significant decreases in HGB statistically significant decreases in HTC in males (at day 29: -17 %, at day 57: - 12 %, at day 92: -10 %) and in females (day 93: -9 %) statistically significant decreases in RBC (day 29 -22 % in males, day 57 -16 % in males, day 92 -16 % in males, day 93 -12 % in females haemosiderin deposition in spleen (10 of 10 male rats,10 of 10 female rats) Pigment deposition in Kupffer cells in liver (8 of 10 male rats, 10 of 10 female rats)	Anonymous 10, 1998

90 day oral study in mice OECD TG 408 GLP Albino mice Crl:CD-1(ICR)BR 10 M, 10 F	Picolinafen technical (Batch CA14113; 97.8 % as) Fed at dietary concentrations of 0, 10.2/12.7, 103.5/148.0, 202.3/279.7, 388.3/577.0 mg/kg bw/d for m/f for 13 weeks	103.5/148.0 mg/kg bw/d: Increase in relative spleen weight extramedullary haematopoiesis in 4 of 10 females and haemosiderin deposition in 10 of 10 males and 10 of 10 females Increase in liver weight, pigment deposition in Kupffer cells in males (1 of 10) 202.3/279.7 mg/kg bw/d Decreases in RBC, statistically not significant, on day 57 and 92 for males and females decreased haemoglobin for males Statistically significant increase in Heinz body formation in males (day 29: 2/1000 RBC compared to 0.8/1000 RBC in control group) Increase in spleen weight, extramedullary haematopoiesis (8 of 10 males, 10 of 10 females) and haemosiderin deposition in all females and males Discoloration of spleen (4 of 10 females) Increase in liver weight, pigment deposition in Kupffer cells in females (9 of 10 compared to 0 of 10 in control group) and males (10 of 10 males compared to 0 of 10 in control group) 388.3/577.0 mg/kg bw/d extramedullary hematopoiesis in spleen (8 of 10 males, 10 of 10 females) and hemosiderin deposition in spleen in all males and all females statistically significant increased reticulocytes in females increase in Heinz body formation males (1.7/1000 RBC compared to 0.8 /1000 RBC in control group on day 29) Decreases in RBC, statistically not significant, on day 57 and 92 for males and females decreased haemoglobin in females and males	Anonymous 9, 1998
90 day oral study in dogs OECD TG 409 GLP Beagle dog 4 M, 4F	Picolinafen technical (Batch CA14113; 97.8 % as) Fed at dietary concentrations of 0, 1.7/1.8, 17.3/20.8, 87.5/92.1 mg/kg bw/d for m/f for 90 days	17.3/20.8 mg/kg bw/d for m/f haemolytic anaemia decreased haemoglobin (-8 % in females), decreased RBC in females (-11 %) changes in thyroid (increased weights, enlarged, hyperplasia and hypertrophy of follicular epithel cells) 87.5/92.1 mg/kg bw/d for m/f Decreased haemoglobin in females (-14 %), decreased RBC in females (- 15 %), decreased HCT in females (-11.5 %)	Anonymous 12, 1999

1 year oral study in dogs OECD TG 452 GLP Beagle dog 4 M, 4 F	Picolinafen technical (Batch CA14113; 97.8 % as) Fed at dietary concentrations of 0, 1.4/1.6, 4.4/5.2, 42.7/47.1 mg/kg bw/d for m/f for at least 1 year	At 42.7 mg/kg bw/d: decrease in haemoglobin (3 months: -9 %, 6 months:-11.4 %), decrease in haematocrit (3 months: -8 %, 6 months: -10 %) in females macroscopic and microscopic changes in thyroid (statistically significant increased relative and absolute organ weight in both sexes, diffuse hypertrophy of follicular epithel cells for all males (slight) and all females (slight to moderate))	Anonymous 13, 1999

<table>
<tr>
<td>2 year study in rats
OECD TG 405
GLP
Reduced survival rate
Sprague Dawley rat Crl:CD® (SD) BR
65 M, 65 F</td>
<td>Picolinafen technical (Batch CA14113; 97.8 % as)
Fed at dietary concentrations of 0, 2.4/3.0, 12.1/15.0, 24.5/31.0 mg/kg bw/d for at least 24 months</td>
<td>At 12.1/15.0 mg/kg bw/d and above: haemolytic anaemia in males and females (decreased red blood cell parameters):
decreased Hb in males (3 months: -7 %, 6 months: -7 %) and females (3 months: -10.8 %, 6 months: -5.4 %, 12 months: -4.3 %)
decreased Ht (3 months: -6 %, 6 months: -6 %) in males and females (3 months: - 11%, 6 months: -5 %)
decreased RBC in males (3 months: -8.3 %, 6 months: -9.3 %) and females (3 months: -13.5 %, 6 months: -6.2 %)
24.5/31.0 mg/kg bw/d
decreased Hb in males (3 months: -9 %, 6 months: -7.9%) and females (3 months: -12 %, 6 months: -9 %, 12 months: -5%)
decreased Ht (3 months: -6 %, 6 months: -6 %) in males and females (3 months: - 9 %, 6 months: -7%, 12 months: -5 %)
decreased RBC in males (3 months: -10.9 %, 6 months: -10.9 % 12 months:-4 %) and females (3 months: -12 %, 6 months: -9.5 %)
spleen: increase in weight
hemosiderin in reticuloendothelial cells of the spleen:
<table>
<tr><td></td><td colspan="4">Males</td><td colspan="4">Females</td></tr>
<tr><td>Brown Pigment: Grading</td><td>0</td><td>50</td><td>250</td><td>500 ppm</td><td>0</td><td>50</td><td>250</td><td>500 ppm</td></tr>
<tr><td>At 12-Months</td><td>(10) [a]</td><td>(10)</td><td>(10)</td><td>(10)</td><td>(10)</td><td>(10)</td><td>(10)</td><td>(10)</td></tr>
<tr><td>Minimal</td><td>5[b]</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Slight</td><td>4</td><td>5</td><td>1</td><td>0</td><td>7</td><td>2</td><td>0</td><td>0</td></tr>
<tr><td>Moderate</td><td>1</td><td>2</td><td>6</td><td>2</td><td>3</td><td>3</td><td>1</td><td>0</td></tr>
<tr><td>Moderately Severe</td><td>0</td><td>2</td><td>3</td><td>8</td><td>0</td><td>5</td><td>9</td><td>10</td></tr>
<tr><td>Unscheduled Deaths</td><td>(42)</td><td>(40)</td><td>(39)</td><td>(40)</td><td>(33)</td><td>(32)</td><td>(37)</td><td>(36)</td></tr>
<tr><td>Minimal</td><td>9</td><td>10</td><td>2</td><td>2</td><td>7</td><td>5</td><td>1</td><td>5</td></tr>
<tr><td>Slight</td><td>10</td><td>12</td><td>11</td><td>8</td><td>4</td><td>11</td><td>10</td><td>4</td></tr>
<tr><td>Moderate</td><td>13</td><td>10</td><td>9</td><td>12</td><td>7</td><td>4</td><td>11</td><td>6</td></tr>
<tr><td>Moderately Severe</td><td>6</td><td>7</td><td>15</td><td>13</td><td>11</td><td>8</td><td>12</td><td>14</td></tr>
<tr><td>Severe</td><td>1</td><td>0</td><td>0</td><td>0</td><td>1</td><td>1</td><td>3</td><td>6</td></tr>
<tr><td>At 24-Months</td><td>(13)</td><td>(15)</td><td>(16)</td><td>(15)</td><td>(22)</td><td>(23)</td><td>(18)</td><td>(19)</td></tr>
<tr><td>Minimal</td><td>8</td><td>9</td><td>7</td><td>4</td><td>2</td><td>7</td><td>4</td><td>2</td></tr>
<tr><td>Slight</td><td>5</td><td>5</td><td>1</td><td>5</td><td>13</td><td>6</td><td>2</td><td>5</td></tr>
<tr><td>Moderate</td><td>0</td><td>0</td><td>7</td><td>5</td><td>5</td><td>9</td><td>4</td><td>3</td></tr>
<tr><td>Moderately Severe</td><td>0</td><td>0</td><td>0</td><td>1</td><td>1</td><td>0</td><td>7</td><td>7</td></tr>
<tr><td>Severe</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>1</td><td>2</td></tr>
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</td>
<td>Anonymous 18, 1999</td>
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18 month study in mice OECD TG 451 GLP CD-1 albino mice 65 M, 65 F	Picolinafen technical (Batch CA14113; 97.8 % as) Fed at dietary concentrations of 0, 6.9/8.2, 68.6/81.0, 137.1/165.8 mg/kg bw/d for m/f for 18 months	68.6 /81.0 mg/kg bw/d: findings indicative of anemia (statistically significant increased reticulocyte counts and MCHC, deposition of pigment in spleen), increased liver weights and hypertrophy at 3 months: statistically significant increase in reticulocytes in males (+96 % compared to control group) Statistically significant increase in MCHC in females (34.8 % compared to 33.5 % in control group) 137.1/165.8 mg/kg bw/d: At 3 months: Statistically significant increase in reticulocytes in males (+83 % compared to control group) Statistically significant increase in MCH in females (+5 %) Statistically significant increase in MCHC in males (+3 %) and females (+4 %)	Anonymous 17, 1999
28 day dermal study in rats OECD TG 410 GLP Sprague Dawley rats 10 M, 10 F	Picolinafen (Batch CA14113; 97.8 % as), administered dermally (mixed with distilled water), 4 cm x 2 cm application site 0, 25, 50, 75, 100, 200, 1000 mg/kg bw/d Treatment for 6 hours per day, 5 days per week, for 4 weeks	Statistically significant decrease in haematocrit (males on day 12: 50 mg/kg bw/d: -2.5 % compared to control, 75 mg/kg bw/d: -7 % compared to control, 100 mg/kg bw/d: - 6 % compared to control, 200 mg/kg bw/d: -10 % compared to control, 1000 mg/kg bw/d: -18 % compared to control; females on day 12: 100 mg/kg bw/d -4 %, 200 mg/kg bw/d: -5 %, 1000 mg/kg bw/d: -10 % compared to control) Statistically significant decrease in haemoglobin (males on day 12: 50 mg/kg bw/d: -5 %, 75 mg/kg bw/d: -8 %, 100 mg/kg bw/d: -7 %, 200 mg/kg bw/d: -11 %, 1000 mg/kg bw/d: -21 % compared to control, females on day 12: 100 mg/kg bw/d: -4 %, 200 mg/kg bw/d: -7 %, 1000 mg/kg bw/d: -14 % compared to control) Statistically significant decrease in erythrocyte count (males on day 12: 75 mg/kg bw/d: -5 %, 100 mg/kg bw/d: -6 %, 200 mg/kg bw/d: -9 %, 1000 mg/kg bw/d: -21 % compared to control, females on day 12: 100 mg/kg bw/d: -4 %, 200 mg/kg bw/d: -8 %, 1000 mg/kg bw/d:-20 % compared to control) 100 mg/kg bw/d: extramedullary haematopoiesis in spleen (8 of 10 males and 5 of 10 females compared to 3 of 10 males and 2 of 10 females in control group), hemosiderin-laden macrophages in 4 of 10 males and 10 of 10 females compared to 1 of 10 males in 2 of 10 females in control group) 200 mg/kg bw/d: increased extramedullary haematopoiesis in spleen (10 of 10 males and 9 of 10 females compared to 3 of 10 males and 2 of 10 females in control group), haemosiderin-laden macrophages (9 of 10 males and 10 of 10 females compared to 1 of 10 males in 2 of 10 females in control group 1000 mg/kg bw/d: increased extramedullary haematopoiesis in spleen (5 of 10 males and 5 of 10 females compared to 3 of 10 males and 2 of 10 females in control group), haemosiderin laden macrophages (4 of 10 males and 5 of 10 females compared to 1 of 10 males in 2 of 10 females in control group)	Anonymous 8, 1999

2 generation study rat GLP Sprague Dawley rats 0-50-250-500 ppm		**P- generation** <u>250 ppm (18/22 mg/kg bw/d for m/f):</u> Statistically significant reduction in haemoglobin (males: -7 %, females: -8 %) Statistically significant reduction in haematocrit (males: -5 %, females: -6 %) Statistically significant reduction in RBC (males: -6%, females:-7 %) Spleen: extramedullary haematopoiesis in 16 of 30 males, 8 of 30 females compared to 0 males, 1 females in control group Spleen: Brown pigment in reticuloendothelial cells in 26 of 30 males (5 mild, 19 slight, 2 moderate) and 26 of 30 females (12 mild, 14 slight) compared to 0 males and 2 females in control group <u>500 ppm (39/44 mg/kg bw/d for m/f)</u> Statistically significant reduction in haemoglobin (males: -9 %, females: -13 %) Statistically significant reduction in haematocrit (males: -6 %, females: -10 %) Statistically significant reduction in RBC (males: -9 %, females: -12 %) Spleen: extramedullary haematopoiesis in 22 of 30 males, 12 of 30 females compared to 0 males and 1 female in control group Spleen: Brown pigment in reticuloendothelial cells in 30 of 30 males (5 mild, 21 slight, 4 moderate) and 30 of 30 females 21 slight, 9 moderate) compared to 0 males and 2 females in control group **F1 generation** <u>250 ppm (ca. 17/27 mg/kg bw/d for m/f)</u> Statistically significant reduction in haemoglobin (males: -3 %, females: -7 %) Spleen: extramedullary haematopoiesis in 9 of 30 males (and 3 of 30 females compared to 1 male and 0 female in control group Spleen: brown pigment in reticuloendothelial cells in 23 of 30 males (16 mild, 7 slight) and 28 of 30 females (20 slight, 8 mild) compared to 1 male and 1 female in control group <u>500 ppm (ca. 34/55 mg/kg bw/d for m/f)</u> Statistically significant reduction in haemoglobin (males: -7 %, females -13 %) Statistically significant reduction in haematocrit (males: -4 %, females:-11 %) Statistically significant reduction in RBC (males: -7 %, females: -15 %) Spleen:extramedullary haematopoiesis in 20 of 30 males and 16 of 30 females compared to 1 male and 0 females in control group Spleen: brown pigment in reticuloendothelial cells 28 of 30 males (2 mild, 25 slight, 1 moderate) and 29 of 30 females (1 mild, 18 slight, 10 moderate) compared to 1 male and 1 female in control group	Anonymous 21, 1999

Developmental toxicity study rat OECD TG 414 GLP Sprague Dawley rats 25 animals per group	Picolinafen technical (Batch CA14113; 97.8 % as) oral gavage day 6 to 19 of gestation first phase: 100, 500, 1000 mg/kg bw/d second phase: 5, 25, 50 mg/kg bw/d	<u>100 mg/kg bw/d maternal animals</u> Statistically significant reduction in haemoglobin: -7 % Statistically significant reduction in haematocrit: -7 % Statistically significant reduction in RBC: -10 % Statistically significant increase in reticulocytes: +29% Spleen: extramedullary haematopoiesis in 22 animals (6 minimal, 11 mild, 5 moderate) compared to 14 animals in control group (8 minimal, 5 mild, 1 moderate) Spleen: hemosiderosis in 18 animals (1 minimal, 12 mild, 5 moderate) compared to 6 in control group (4 minimal, 2 mild) <u>500 mg/kg bw/d maternal animals</u> Statistically significant reduction in haemoglobin: -7 % Statistically significant reduction in haematocrit: -8 % Statistically significant reduction in RBC: -18 % Statistically significant increase in reticulocytes: +82 % Spleen: extramedullary haematopoiesis in 24 animals (3 minimal, 11 mild, 10 moderate) compared to 14 animals in control group (8 minimal, 5 mild, 1 moderate) Spleen: hemosiderosis in 25 animals (0 minimal, 5 mild, 20 moderate) compared to 6 in control group (4 minimal, 2 mild) <u>1000 mg/kg bw/d maternal animals</u> Statistically significant reduction in haemoglobin: -8 % Statistically significant reduction in haematocrit: -10 % Statistically significant reduction in RBC: -22 % Statistically significant increase in reticulocytes: +114 % Spleen: extramedullary hematopoiesis in 24 animal (3 minimal, 9 mild, 12 moderate) compared to 14 animals in control group (8 minimal, 5 mild, 1 moderate) Spleen: haemosiderosis in 25 animals (1 minimal, 5 mild, 19 moderate) to 6 in control group (4 minimal, 2 mild) Spleen: mild inflammation in capsule in 1 animal (0 in control group)	Anonymous 18, 1999

Developmental toxicity study rabbit OECD TG 414 GLP New Zealand White Rabbits 25 females per group	Picolinafen technical (Batch CA14113; 97.8 % as) Oral gavage 5, 20, 50 mg/kg bw/d Day 6 to 28 of gestation	20 mg/kg bw/d Statistically significant reduction in RBC (-11 %) Reduction in HB (-7 %) Reduction in HCT (-8 %) Spleen: haemosiderin deposition increased (20 of 25 animals, 4 minimal, 9 mild, 6 moderate, 1 marked) compared to 10 animals in control group (6 minimal, 3 mild, 1 moderate) 50 mg/kg bw/d Statistically significant reduction in RBC (-27 %) Reduction in HB (-14 %) Reduction in HCT (-15 %) Spleen: haemosiderin deposition increased (20 of 25 animals, 7 minimal, 7 mild, 6 moderate) compared 10 animals in control group (6 minimal, 3 mild, 1 moderate) Extramedullary haematopoiesis in spleen in 13 of 25 animals compared to 1 of 25 animals in control group	Anonymous 11, 1998

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

Anaemia was noted in both the 28-day (Anonymous 15, 1993) and 13-week rat studies (Anonymous 10, 1998), as evidenced by changes in haematological parameters, increased absolute and relative spleen and liver weights, and microscopic changes in the bone marrow, kidney, and/or spleen and liver. Additionally, decreased food consumption, mean body weights and weight gains were noted in the 13-week rat study beginning at weeks 4 to 6.

Anaemia was also noted in both the 28-day (Anonymous 14, 1998) and 13-week mouse studies (Anonymous 9, 1998), as evidenced by changes in haematological parameters, increased absolute and relative spleen and liver weights, and microscopic changes in the spleen and liver. Additionally, hepatocellular hypertrophy was noted in both studies. In the 13-week study, decreased food consumption and mean body weights and weight gains were observed.

In the 28-day dog study (Anonymous 11, 1998), anaemia was noted, as evidenced by changes in haematological parameters. Increased absolute and relative thyroid/parathyroid weights and thyroid follicular cell hypertrophy and hyperplasia were also noted. Additionally, increased serum cholesterol was noted, and decreased mean body weights and/or weight gains observed.

In the 2 dog studies at 90 days (i.e. 90-day dietary study, Anonymous 12 1999, and at the 90-day time point in the one-year dog study, Anonymous 13 1999), anaemia was noted, as evidenced by changes in haematological parameters. Increased absolute and relative thyroid/parathyroid weights and thyroid follicular cell hypertrophy and hyperplasia were also noted in the 90-day and the one-year studies. Decreased mean body weights and/or weight gain was also observed in the 90-day study.

Results from a 28-day dermal toxicity study conducted in Sprague-Dawley rats (Anonymous 8, 1999) with Picolinafen technical revealed a anaemia, as evidenced by changes in haematological parameters, beginning on Study Day 5. Additionally, increases in absolute and/or relative spleen weights and microscopic changes in the spleen were noted.

Similar findings regarding induction of anaemia were observed in the multigeneration study in rats (Anonymous 21, 1999) at dose levels of 18.82/22.16 mg/kg bw/d and 38.76/44.07 mg/kg bw/d, in the

developmental toxicity study in rats (Anonymous 18, 1999) at 100, 500 and 1000 mg/kg bw/d and in the developmental toxicity study in rabbits (Anonymous 11, 1998) at 20 and 50 mg/kg bw/d.

Group Mean Hematology Values in 90-day rat study (Anonymous 10, 1998)

Males					
Parameter	Measurement Interval	0 ppm	80 ppm	400 ppm	800 ppm
HB (g/dl)	57 days	15.0	14.9	13.7*	13.0*
	92 days	15.7	15.5	14.3*	13.9*
HCT (%)	29 days	48.9	46.9	42.9	40.8*
	57 days	42.3	41.6	39.2*	37.2*
	92 days	45.6	45.0	41.9*	41.2*
RBC (10^6/mm^3)	29 days	7.6	7.2	6.5*	5.9*
	57 days	7.3	7.1	6.6*	6.1*
	92 days	8.1	7.8	7.2*	6.8*
Females					
Parameter	Measurement Interval	0 ppm	80 ppm	400 ppm	800 ppm
HB (g/dl)	57 days	14.5	14.4	13.8*	13.1*
	93 days	15.2	15.0	14.0*	13.5*
HCT (%)	93 days	44.5	44.6	42.3*	40.6*
RBC (10^6/mm^3)	93 days	7.2	7.2	6.7*	6.4*

HB = hemoglobin; RBC = erythrocyte count; HCT = hematocrit;
* (= 0.05) significantly different from control (Dunnett`s method)

Incidence of histopathological findings in thyroid in 90 day dog study (Anonymous 12, 1999)

Thyroid					
		0 ppm	50 ppm	500 ppm	2500 ppm
Hypertrophy					
Total	Male	0	0	3	4
	Female	0	0	3	4
Trace	Male	0	0	3	0
	Female	0	0	3	0
Mild	Male	0	0	0	2
	Female	0	0	0	1
Moderate	Male	0	0	0	2
	Female	0	0	0	2
Severe	Male	0	0	0	0
	Female	0	0	0	1
Hyperplasia, diffuse					
Total	Male	0	0	0	4
	Female	0	0	0	4
Trace	Male	0	0	0	2
	Female	0	0	0	1
Mild	Male	0	0	0	1
	Female	0	0	0	2
Moderate	Male	0	0	0	1
	Female	0	0	0	0
Severe	Male	0	0	0	0
	Female	0	0	0	1
Epithelium					
Flattened	Male	4	4	1	0
	Female	4	4	1	0
Low cuboidal	Male	0	0	3	0

	Female	0	0	3	0
High cuboidal	Male	0	0	0	2
	Female	0	0	0	2

Table 33: Extrapolation of equivalent effective dose for toxicity studies of greater or less duration than 90 days

Study reference	**Effective dose (mg/kg/d), at which haemolytic anemia or pathological alterations in thyroid is seen**	**Length of exposure**	**Extrapolated effective dose when extrapolated to 90-day exposure**	**Classification supported by the study**
28 day rat Anonymous 15, 1993	107 mg/kg bw/d (haemolytic anemia)	28 days	36 mg/kg bw/d	yes
28 day mouse Anonymous 14. 1998	227 mg/kg bw/d (haemolytic anemia)	28 days	76 mg/kg bw/d	yes
28 day dog Anonymous 11, 1998	44 mg/kg bw/d (alterations in thyroid)	28 days	15 mg/kg bw/d	yes
90 day rat Anonymous 10, 1998	32 mg/kg bw/d (haemolytic anemia)	90 days	32 mg/kg bw/d	yes
90 day mouse Anonymous 9, 1998	104 mg/kg bw/d (haemolytic anemia)	90 days	104 mg/kg bw/d	yes (borderline)
90 day dog Anonymous 12, 1999	17 mg/kg bw/d (alterations in thyroid)	90 days	17 mg/kg bw/d	yes
1 year dog Anonymous 13, 1999	43 mg/kg bw/d (alterations in thyroid and anemia)	1 year	86 mg/kg bw/d	yes
2 year rat Anonymous 18, 1999	12 mg/kg bw/d (haemolytic anemia)	2 years	24 mg/kg bw/d	yes
18 month mice Anonymous 17, 1999	69 mg/kg bw/d	18 months	138 mg/kg bw/d	no
2- generation rat	18 mg/kg bw/d	10 weeks prior to mating period, during mating period (14 days) and during post-mating period (gestation, lactation, post-weaning period)	18 mg/kg bw/d	yes
Developmental toxicity rat	100 mg/kg bw/d (haemolytic anemia)	Day 6 to day 19 of gestation		yes
Developmental toxicity rabbit	20 mg/kg bw/d (haemolytic anemia)	Day 6 to day 28 of gestation		yes

10.12.2 Comparison with the CLP criteria

Table 34: Selected toxicological results (at dose levels below the guidance values) in comparison with criteria of specific target organ toxicity – repeated exposure

Toxicological data	**CLP criteria**
28-d oral studies in rats: 107/119 mg/kg bw/d: haematology (haemoglobin and RBC ↓, methaemoglobin and Heinz bodies ↑), clinical chemistry (plasma bilirubin ↑, spleen (incr. extramedullary haematopoiesis, haemosiderin deposition), bone marrow (incr. erythropoiesis), liver (erythropoietic foci, haemosiderin deposition in Kupffer cells) **28-d oral studies in mice:** 227.2/234.9 mg/kg bw/d: anaemia (incr. extramedullary haematopoiesis and brown pigment deposition in spleen) **28-d oral studies in dogs:** 47.7/43.9 mg/kg bw/d, 90.4/71.4 mg/kg bw/d, 313.4/248.5 mg/kg bw/d: serum cholesterol, thyroid (wt ↑, diffuse hyperplasia and hypertrophy) **90-d oral studies in rats:** 32.2/35.1 mg/kg bw/d, 65.4/69.0 mg/kg bw/d: haematology (haemoglobin, haematocrit and RBC ↓), spleen (haemosiderin deposition), liver (haemosiderin deposition in Kupffer cells) **90-d oral studies in mice:** 103.5/148.0 mg/kg bw/d: spleen (incr. extramedullary haematopoiesis, haemosiderin deposition) **90-d oral study in dogs:** 17.3/20.8 mg/kg bw/d; 87.5/92.1 mg/kg bw/d: haematology (haemoglobin, haematocrit and RBC ↓), alterations in thyroid **1-yr oral study in dogs:** 43 mg/kg bw/d: decrease in haemoglobin, haematocrit and pathological changes in thyroid **2-yr study in rats:** 12.1/15.0 mg/kg bw/d: haematology (haemoglobin, haematocrit and RBC ↓), spleen (haemosiderin deposition) **18-mo study in mice:** Effect levels were above guidance values **28-d dermal studies in rats:** 75 mg/kg bw/d: haematology (haemoglobin, haematocrit and RBC ↓) 100 mg/kg bw/d, 200 mg/kg bw/d: haematology (haemoglobin, haematocrit and RBC ↓), spleen (incr. extramedullary haematopoiesis, haemosiderin deposition) **2 generation study rats:** 18 mg/kg bw/d: haematology (haemoglobin, haematocrit and RBC ↓), extramedullary haematopoiesis and haemosiderosis in spleen	Category 1 (H372): Substances that have produced significant toxicity in humans or that, based on evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Equivalent guidance values for different study durations: Oral, rat: 28-day: ≤ 30 mg/kg bw/d 90-day: ≤ 10 mg/kg bw/d 1-yr: ≤ 2.5 mg/kg bw/d 2-yr: ≤ 1.25 mg/kg bw/d Dermal: 28-day: ≤ 60 mg/kg bw/d 90-day: ≤ 20 mg/kg bw/d Category 2 (H373): Substances that, based on evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) based on observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below in order to help in classification. In exceptional cases, human evidence can also be used to place a substance in Category 2. Equivalent guidance values for different study durations: Oral, rat: 28-day: 30 < C ≤ 300 mg/kg bw/d 90-day: 10 < C ≤ 100 mg/kg bw/d 1-yr: 2.5 < C ≤ 25 mg/kg bw/d 2-yr: 1.25 < C ≤ 12.5 mg/kg bw/d Dermal:

2 generation study rats: 18 mg/kg bw/d: haematology (haemoglobin, haematocrit and RBC ↓), extramedullary haematopoiesis and haemosiderosis in spleen	28-day: 60 < C ≤ 600 mg/kg bw/d 90-day: 20 < C≤ 200 mg/kg bw/d 28-day: 60< C ≤ 600 mg/kg bw/d 90-day: 20 < C ≤ 200 mg/kg bw/d
Developmental toxicity study rabbit: 20 mg/kg bw/d: haematology (haemoglobin, haematocrit and RBC ↓), haemosiderosis in spleen	

10.12.3 Conclusion on classification and labelling for STOT RE

10.12.3.1 Anemia

Reduced haemoglobin concentration and red blood cell count are repeatedly seen in studies performed with Picolinafen leading to the conclusion that the substance causes anaemia. The effects (haematological findings and corroborating histological findings in spleen, liver, kidney, bone marrow) are described in detail in Table 35. Further classification into haemolytic anemia, which is usually caused by accelerated destruction of mature red cells, is supported by findings, which are summarized in the following table.

Findings being indicative of haemolytic anemia:

Pathological alteration	**Study reference**
occurrence of Heinz bodies (consisting of precipitated haemoglobin that is attached to the internal surface of erythrocyte membranes causing red blood cell lysis)	28 day oral rat study, Anonymous 15, 1993 28 day oral mice study, Anonymous 14, 1998 90 day mice study, Anonymous 10, 1998
Methaemoglobinemia	28 day oral rat study, Anonymous 15, 1993
haemosiderosis (deposition of hemosiderin) in the spleen	28 day oral rat study, Anonymous 15, 1993 28 day oral mouse study, Anonymous 14, 1998 90 day oral rat study, Anonymous 10, 1998 90 day mice study, Anonymous 9, 1998 2 year rat study, Anonymous 18, 1999 28 day dermal rat study, Anonymous 8, 1999
Hemosiderosis in the liver (being indicative of intravascular hemolysis)	28 day oral rat study, Anonymous 15, 1993 28 day oral mouse study, Anonymous 14, 1998 90 day oral rat study, Anonymous 10, 1998 90 day mice study, Anonymous 9, 1998
Haemosiderosis in the kidney	28 day oral rat study, Anonymous 15, 1993
increased MCHC (being indicative of massive intravascular haemolysis)	18 month mice study Anonymous 10, 1998
Hyperbilirubinemia	28 day oral study in rats, Anonymous 15, 1993
Splenomegaly (can be indicative of increased degradation of erythrocytes)	28 day oral rat study, Anonymous 15, 1993 90 day mice study, Anonymous 9, 1998
focal capsular inflammation /proliferation in spleen	28 day oral rat study, Anonymous 15, 1993 rabbit developmental toxicity study 19, 1998

increased erythropoiesis in the bone marrow	28 day oral rat study, Anonymous 15, 1993

After dermal administration of 200 mg/kg bw/d to rats, haemoglobin levels were reduced by more than 10 % after 12 and 27 days in males and after 27 days in females. These findings were corroborated by increased haemosiderosis (1 of 10 males "traces" and 9 of 10 males "mild" compared to 9 of 10 males "traces" and 1 of 10 males "mild" in control group) and 10 of 10 females (10 of 10 females "mild" compared to 8 of 10 females "traces" and 2 of 10 females "mild" in control group) and increased extramedullary hematopoiesis in spleen (10 of 10 males "mild" compared to 7 of 10 males "traces" and 3 of 10 males "mild" and 1 of 10 females "traces", 8 of 10 females "mild", 1 of 10 females "moderate" compared to 8 of 10 females "traces", 2 of 10 females "mild" in control group).

In studies with oral administration to rats for periods of up to two years (including the developmental toxicity and 2-generation studies in rats), increased severity and incidences of haemosiderin pigment deposition in spleen were described; haemoglobin levels were reduced by approximately 10 % reaching in several studies levels above 10 % reduction. Occasionally these findings were corroborated by haemosiderin deposition in liver. The histological findings were described as moderately severe to severe.

In the developmental toxicity study in rabbits, haemoglobin levels were reduced by more than 10 % and haemosiderin deposition occurred in increased incidence and increased severity in animals dosed with 50 mg/kg bw/d.

Also in the available short-term studies in mice, increased severity and increased incidences of pigment deposition in spleen were observed. Haemoglobin levels were reduced by less than 10 %.

In the sub-chronic/chronic studies in dogs, haemoglobin levels were reduced by more than 10 %, but these findings were not corroborated by pigment deposition in spleen.

Signs of anaemia were supported by compensatory increase in extramedullary haematopoiesis in most studies in rats, mice and rabbits.

Anaemia was seen in studies with oral and dermal administration. No information or data is available for inhalative administration.

The findings described above, were generally seen at dose levels consistent with the guidance values for category 2.

During the Peer Review on the active substance Picolinafen the notifier commented on the proposal to classify in STOT RE 2 (EFSA Peer Review Report on Picolinafen October 2015)

"In all studies anaemia was always reported as 'slight', 'moderate' at most, and lower LOAELs obtained in studies of longer duration are a result of the different dosing spacing applied. In the 2-y combined chronic toxicity study in rats, anaemia was evident at 3 and 6-month endpoints, but not at the 1-y and terminal (2-y) examination".

The Guidance on the application of CLP criteria (Version 5.0, July 2017) states in section 3.9.2.5.2:

"The guidance developed for classification of substances inducing haemolytic anaemia according to 67/548/EEC (Muller A. et al., 2006) cannot directly be used under CLP because of the changes in criteria (see CLP Annex I, 3.9.2.7.3 c and 3.9.2.8.b, d). The major criterion for haemolytic anaemia changed:

- *From "Any consistent changes in haematology which indicate severe organ dysfunction."*
- *To "Any consistent and significant adverse changes in haematology."*

This indicates that less adverse effects are considered for classification according to CLP. This is consistent with the changes in the other criteria for classification for repeated exposure.

Adaptation towards the criteria according to CLP results in the following guidance:

"It is evident that anaemia describes a continuum of effects, from sub-clinical to potentially lethal in severity. Overall, the interpretation of study findings requires an assessment of the totality of findings, to judge whether they constitute an adaptive response or an adverse toxicologically significant effect. If a haemolytic substance induces one or more of the serious health effects listed as examples below within the critical range of doses, classification is warranted. It is sufficient for classification that only one of these criteria is fulfilled. […]"

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

Example:

- *Marked increase of haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥10 %) in a 28-day study.*
- *Significant increase in haemosiderosis in the spleen, liver or kidney in combination with microscopic effects like necrosis, fibrosis or cirrhosis.*

The observed findings correlate to those mentioned in the guidance as examples (paleness of extremities, paleness of organs, reduction in Hb up to 28 % (28-day oral dog study), MetHb increase, fibrosis in spleen) and were observed in the critical dose range.

In addition, aniline and structurally related compounds are classified as STOT RE 2 for haemolytic effects, as they provoked inflammatory and capsular lesions of spleen associated with haemosiderin deposition and red pulp comparable to those seen in studies with Picolinafen. Thus, it is concluded that this syndrome of changes may have similar pathogenesis to those produced by other aromatic amines, which is due to chemically-mediated erythrocyte toxicity and subsequent damage to the spleen by accumulation of damaged cells in this organ, deposition of erythrocytic debris, which might catalyse tissue-damaging free radical reactions and induction of hyperplasia of the spleen (Bus and Popp 1987, Perspectives on the mechanism of action of the splenic toxicity of aniline and structurally-related compounds, Fd Chem Toxic Vol 25, No 8, pp 619-626).

10.12.3.2 Thyroid

Furthermore, pathological changes in thyroid (hyperplasia, hypertrophy) were observed in several dog studies at dose levels below the guidance values for Cat.2. In the 28 day dog study (Anonymous 11, 1998), diffuse hyperplasia of thyroid at 48/44 mg/kg bw/d (m/f) was "moderate" in one male and "severe" in one male animal, and "mild" in one female and "moderate" in one female animal. "Severe" diffuse hyperplasia was seen at all next higher dose levels in all animals.

In the 90 day dog study (Anonymous 12, 1999), hypertrophy of thyroid ("trace") was seen at 17 respectively 20 mg/kg bw/d in 3 of 4 male and 3 of 4 female animals, at 87.5 respectively 92 mg/kg bw/d in 4 of 4 male animals (2 "mild", 2 "moderate") and 4 of 4 female animals (1 "mild", 2 "moderate", 1 "severe").

In the 1 year dog study (Anonymous 13, 1999), 2 of 4 females showed "moderate" follicular cell hypertrophy of thyroid and 2 of 4 females showed "slight" follicular cell hypertrophy, whereas 4 of 4 male animals showed "slight" follicular cell hypertrophy of thyroid at 47 (females) respectively 43 (males) mg/kg bw/d. Follicular epithelium was "low cuboidal" in 3 of 4 males and 1 of 4 females and "high cuboidal" in 1 of 4 males and 3 of 4 females, whereas the cells were flat in the control group. Follicular cell hyperplasia was seen in 2 females (1 minimal, 1 slight) at this dose level.

The dose-dependent increase in hyperplasia of thyroid at dose levels below the guidance values for Cat. 2 is considered to be adverse and severe enough to justify classification.

In summary, it is proposed to classify Picolinafen with STOT RE 2 (H373, blood and thyroid).

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

Summary of the Dossier Submitter's proposal

The DS provided in Table 32 of CLH report the summaries of the repeated-dose studies on picolinafen which were conducted:

- in rats (28-day dietary, 28-day dermal, 90-day dietary, 2-year dietary combined chronic toxicity/ carcinogenicity study, 2-generation reproductive dietary study, developmental toxicity study),
- in mice (28-day dietary, 90-day dietary, 18-month dietary),
- in dogs (28-day dietary, 90-day dietary, 1-year dietary) and
- in rabbits (developmental toxicity study).

Reduced haemoglobin concentration and red blood cell count (RBC) were repeatedly seen in studies performed on rats, mice, dogs and rabbits with picolinafen leading to the conclusion that the substance causes anaemia. Increased absolute and relative thyroid/parathyroid weights and thyroid follicular cell hypertrophy and hyperplasia were also seen in repeated dose toxicity studies performed on dogs.

Based on results of these studies, the DS has proposed a classification of picolinafen for STOT RE 2, H373: May cause damage to organs (blood and thyroid) through prolonged or repeated exposure.

Comments received during consultation

One industrial organisation disagrees with the proposed classification as STOT RE 2 based on anaemia, however noting that the 28-day dermal repeated exposure to picolinafen induced anaemia (21%) above the guidance value of 20% in rats, but only at 1000 mg/kg bw/d. More precisely, this industrial organisation commented "In all other studies, the haemoglobin (Hb) deficit (or Hb+MetHb (methaemoglobin) deficit) does not achieve 20%; there is no serious pathology to trigger consideration of a 10% guidance value. Haemosiderin pigmentation does not achieve a "severe" grade. The tendency to anaemia is well-compensated in all studies. The criteria for STOT RE 2 are (para 3.9.1.3 Regulation 1272/2008) "*toxicologically significant changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism.*"

In response, the DS noted that the guidance value for STOT RE 2 in the 28-day dermal rat study is ≤ 600 mg/kg bw/d. As anaemia (e.g. reduction in Hb by 11%) was accompanied by corresponding effects including extramedullary haematopoiesis in spleen, haemosiderosis in spleen, decrease in haematocrit (HCT) value, decrease of erythrocyte count at doses below this guidance value, the data are considered to support

the classification proposal. The DS further noted that haemosiderin pigmentation was reported as "severe" in 7 males and as "moderate" in 3 males and "severe" in 9 females and "moderate" in 1 female at 107/119 mg/kg bw/d (m/f) in the 28-day oral study in rats. In the 2-year study in rats, moderately severe haemosiderin pigmentation below the guidance value of 12.5 mg/kg bw/d was reported for 3 out of 10 males and 9 out of 10 females at 12-month interval. Severe haemosiderin pigmentation at this dose level was observed in 3 female animals (unscheduled death) and 1 female at 24 months.

The DS considered that the key criteria for STOT RE classification given in Regulation 1272/2008 (point 3.9.2.7.3.(c)) "*any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters*" are fulfilled by effects observed in 28-day oral dog study such as reduction in Hb by 19% in males was seen at 90 mg/kg bw/d and by 28% at 250 mg/kg bw/d. The DS also noted that in the assessment of effects for classification as STOT RE, the following requirement, defined in point 3.9.1.4. of Regulation 1272/2008, should be considered: "*Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs*." Such generalised changes of a less severe nature involving several organs were seen in the following repeated dose toxicity studies of picolinafen:

- reduction in Hb by 19% (at 90 mg/kg bw/d) accompanied by marked increase of haemosiderosis in the spleen, liver and kidney, focal capsular inflammation and capsular fibrotic proliferation in 28-day oral rat study;
- haemosiderosis in spleen, extramedullary haematopoiesis in spleen, formation of Heinz bodies (at < 300 mg/kg bw/d) in 28-day oral mice study;
- reduction in Hb by 28% (at 249 mg/kg/d) in 28-day dog study;
- reduction in Hb by 12% (at 32 mg/kg bw/d) accompanied by haemosiderosis in spleen and liver in 90-day oral rat study;
- haemosiderosis in spleen (at 103.5 mg/kg bw/d) in 90-day oral mice;
- reduction in Hb by 11% (at 200 mg/kg bw/d) accompanied by extramedullary haematopoiesis and haemosiderosis in spleen in 28-day dermal rat.

Based on the above evidence the DS was of the opinion that classification of picolinafen as STOT RE 2, H373 is justified.

Additional key elements

In the RAC plenary meeting one industry representative repeated the opposing view on STOT RE classification for blood system effects (as per the written comments). In addition, this representative also disagreed with the proposal to classify STOT RE for thyroid effects.

Assessment and comparison with the classification criteria

There are three 28-day oral repeated toxicity studies with picolinafen, in rats, in mice and in dogs, three 90-day oral repeated toxicity studies in rats, in mice and in dogs, 1-year

oral study in dogs, 2-year oral study in rats and 18-month oral study in mice. In addition, the results of one 2-generation study in rats, and two prenatal developmental toxicity studies in rats and rabbits were taken into account.

These studies demonstrate that the most sensitive cells are the erythrocytes where picolinafen induces haemolysis, leading to their premature destruction, reduction of Hb level in blood, increased medullary and extramedullary haematopoiesis, increased percentage of reticulocytes in blood, and deposition of haemosiderin in spleen and liver. The reduction in haemoglobin, red blood cell count (RBC) and haematocrit (HCT) are typical symptoms of haemolytic anaemia, which is an adverse but reversible effect.

According to point 3.9.2.7.3 (c) of Regulation 1272/2008 "*any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters"* are considered to support classification for STOT RE.

Point 3.9.1.4. of Regulation 1272/2008 states: "*Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs*."

According to Guidance on the Application of the CLP Criteria Version 5.0 – July 2017, a reduction in Hb at ≥ 20%, or marked increase of haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥ 10%) in a 28-day study is sufficient for classification.

On the other hand, according to point 3.9.2.8.1 (b) of Regulation 1272/2008 "*small changes in clinical biochemistry, haematology or urinalysis parameters and/or transient effects, when such changes or effects are of doubtful or minimal toxicological importance"* are considered not to support classification for specific target organ toxicity following repeated exposure.

Since 90-day or longer studies are considered to be most appropriate for assessment of repeated dose toxicity, a comparison of the observed effects with classification criteria starts with studies of that duration.

90-days or longer studies

Guidance values (GVs) for 90-day oral studies for STOT RE 1 equal to C ≤ 10 mg/kg bw/d or for STOT RE 2 amounting 10 < C ≤ 100 mg/kg bw/d can be found in Annex I to CLP, tables 3.9.2 and 3.9.3. For studies of greater or lesser durations, GVs have been extrapolated using Haber's rule (C x t=constant) (see 3.9.2.9.5, Annex I, CLP).

<u>Oral exposure</u>

1. In a 90-day study in rats (Anonymous 10, 1998; OECD TG 408, GLP), the animals were given picolinafen at dietary concentrations of 0, 80, 400, or 800 ppm for 13 consecutive weeks, corresponding to dose levels of 0, 6.4, 32.2 and 65.4 mg/kg bw/d for males and 0, 6.8, 35.1 and 69.0 mg/kg bw/d for females.

   No treatment-related mortalities or clinical signs of toxicity were observed during the 13-week study period. Reduced RBC up to 16% and Hb concentration up to 11.5% were observed at exposure level of 35.1/69 mg/kg bw/d (females) and of 32.2/65.4 mg/kg bw/d (males). A decrease of haemoglobin in blood at the end of exposure was by 9% in males and 8% in females exposed at 32.2/35.1 mg/kg

bw/d and by 11.5% in males and 11% in females in animals exposed at dose of 65.4/69.0 mg/kg bw/d. Increases in the incidences of haemosiderin deposition in liver Kupffer cells were noted for males and females at 32.2/35.1 and 65.4/69.0 mg/kg bw/d compared to controls (7 out of 10 males and 8 out of 10 females at 32.2/35.1 mg/kg bw/d and 8 out of 10 males and 10 out of 10 females at 65.4/69.0 mg/kg bw/d versus 0 out of 10 for both males and females in the control group). In the spleen there was haemosiderin deposition, and the severity of this haemosiderin deposition was increased dose-related in males and females at 32.2/35.1 and 65.4/69.0 mg/kg bw/d compared to controls. No other histopathological changes in liver and spleen and no occurrence of no extramedullary haematopoiesis were reported.

Conclusion: Reduction of haemoglobin level in blood up to 11.5% in male and female rats exposed orally to picolinafen at doses 65.4/69.0 mg/kg bw/d, reduction of RBC up to 16% in males at the end of exposure at 65.4 mg/kg bw/d, as well as an increase in incidence and severity of haemosiderin deposition in liver Kupffer cells and in the spleen are regarded as adverse effects on erythrocytes and organs (liver, spleen) involved in the generation and removal of blood cells, and meeting the classification criteria. It is noted that more severe effects in blood, liver and spleen may be expected to occur below the cut-off value of 100 mg/kg bw/d. Therefore, a classification in STOT RE 2 (blood system) is warranted based on this study.

2. In a 90-day study in mice (Anonymous 9, 1998; OECD TG 408, GLP), picolinafen was fed to five groups of each 10 males and 10 females CD-1 albino mice at dietary concentrations of 0, 50, 500, 1000 or 2000 ppm for 13 weeks corresponding to dose levels of 10.2/12.7, 103.5/148.0, 202.3/279.7 and 388.3/577.0 mg/kg bw/d in males/females.

   No treatment-related mortalities or treatment-related clinical signs of toxicity were observed during the 13-week study period. In mice treated with dietary dose levels of 50 ppm no adverse findings were reported. In mice exposed at 500 ppm histological findings were indicative of anaemia (in spleen increased incidence of extramedullary haematopoiesis in 4 out of 10 females as well as haemosiderin deposition in 10 out of 10 males and 10 out of 10 females and increase in liver weight, pigment deposition in Kupffer cells in males).

   In mice exposed at 1000 ppm, the following effects were observed:

   - in blood; decreases in RBC (statistically not significant on day 57 and 92 for males and females), decreased haemoglobin for males, statistically significant increase in Heinz body formation in males (day 29: 2/1000 RBC compared to 0.8/1000 RBC in control group). Data on actual reduction of haemoglobin concentration in blood were not provided.
   - in spleen; increase in organ weight and extramedullary haematopoiesis (8 out of 10 males, 10 out of 10 females) as well as haemosiderin deposition in all females and males,
   - in liver; increase in organ weight, pigment deposition in Kupffer cells in

females (9 out of 10 compared to 0 out of 10 in control group) and males (10 out of 10 males compared to 0 out of 10 in control group).

Conclusion: The data obtained in this study indicate that picolinafen induces slight haemolytic anaemia in mice and affects organs involved in the generation (extramedullary haematopoiesis in spleen) and removal of blood cells (pigment deposition in liver of males and spleen of females), but severity of the described effects at doses close to or below the guidance value of 100 mg/kg bw/d does not meet classification criteria for STOT RE 2.

3. In a 90-day study in dogs (Anonymous 12, 1999; OECD TG 408, GLP), picolinafen was fed to four groups of each 4 male and 4 female Beagle dogs at dietary concentrations of 0, 50, 500, or 2500 ppm for 90 days (equal to 1.7/1.8, 17.3/20.8, 87.5/92.1 mg/kg bw/d, respectively for males and females). There were no mortalities observed during the 90-day study period. Clinical observations, food consumption, ophthalmology evaluations and urinalysis data did not reveal any adverse effects of treatment. Haemolytic anaemia (reduction of Hb in blood by 8.2% and RBC by 11.1%) was noted for females at 500 ppm. Haemoglobin in blood was lowered by 13.5%, RBC by 11.1% and HCT by 11.5% in comparison with control animals in female dogs exposed at 2500 ppm at study termination. No data on Hb, RBC or HCT were provided for male dogs. Macroscopic findings at necropsy included enlarged thyroid glands for males and females at 2500 ppm. Microscopically, diffuse hyperplasia and hypertrophy were noted in the thyroid follicular cells for all males and females at 2500 ppm, as compared to 0 out of 4 males and 0 out of 4 females in the control group. Hyperplasia was characterised as trace for 3 out of 8 animals, mild for 3 out of 8 animals, moderate for 1 out of 8 animals and severe for 1 out of 8 animals at 2500 ppm. Hypertrophy was characterised as mild for 3 out of 8 animals, moderate for 4 out of 8 animals and severe for 1 out of 8 animals at 2500 ppm. At 500 ppm, changes in the thyroid gland were limited to trace hypertrophy for 3 out of 4 males and 3 out of 4 females. The follicular epithelium of thyroids from control animals and from one male and one female at 500 ppm had a flattened cuboidal appearance, while the follicular epithelium of thyroids from the 6 animals with trace hypertrophy at 500 ppm had a low cuboidal appearance and the follicular epithelium of thyroids from 2500 ppm animals (mild to severe) had a high cuboidal to columnar appearance.

   Conclusion: Slight anaemia as shown by reduction of haemoglobin level, RBC and HCT in blood slightly more than 10% in female dogs exposed at 2500 ppm (92.1 mg/kg bw/d) not associated with increased haemosiderin deposits in liver Kupffer cells and in the spleen is not sufficiently adverse effect to meet criteria for classification to STOT RE 2 (blood system).

   The thyroid changes (increased weight and follicular cells hypertrophy and hyperplasia as observed in males and females at 2500 ppm) could be considered to occur as a result of repeated stimulation of this organ caused by the well-characterised negative feedback control effect arising from plasma T4 depletion, although no data on thyroid hormones in blood were provided. Slight, not

statistically significant increases in absolute and relative liver weights were noted for males and females at 2500 ppm compared to controls, however, no corroborating microscopic or biochemical findings in the liver were reported. Therefore, hyperplasia and hypertrophy of thyroid follicular cells cannot be explained by increased activity of liver microsomal enzymes and depletion of thyroid hormones in blood. Considering that mean absolute and relative thyroid/parathyroid weights in male and female dogs at 2500 ppm (87.5/92.1 mg/kg bw/d) were more than two times larger than in control animals it is considered that this effect is adverse and warrants classification as STOT RE 2 (thyroid).

4. In one-year dietary toxicity study in dogs (Anonymous 13, 1999; OECD TG 408, GLP), picolinafen was given to four groups of each 4 male and 4 female Beagle dogs at dietary concentrations of 0, 50, 150, or 1500 ppm at least one year (equal to 1.4/1.6, 4.4/5.2 or 42.7/47.1 mg/kg bw/d, respectively for males and females).

   There were no mortalities observed during the one-year study period. There were no treatment-related clinical signs of toxicity or treatment-related changes in food consumption, ophthalmology or urinalysis data.

   A slight haemolytic anaemia, characterised by not statistically significant decreases in haemoglobin, HCT and red blood cells at 3 and 6 months, was noted for females at 1500 ppm compared to controls. After three months of exposure at the same highest dose, haemoglobin concentration in blood of female dogs was reduced by 9% and after 6 months of exposure by 11.4%. Additionally, a slight increase in reticulocytes was observed in females in the 1500 ppm group at 3, 6, and 9 months (statistically significant at 9 months only) compared to controls. No changes in haematology parameters were noted at study termination.

   Statistically significant increases in mean absolute and relative thyroid/parathyroid weights were noted for males and females, but only at 1500 ppm compared to controls. Macroscopic findings at necropsy showed enlarged thyroid glands for all males and females at the same dose. Microscopically, diffuse hypertrophy of thyroid follicular epithelial cells was noted for all males and females at 1500 ppm, as compared to 0 out of 4 control males and 0 out of 4 control females. The severity of this finding was diagnosed as slight for males and slight-to-moderate for females. The follicular epithelium of thyroids from control animals had a flattened cuboidal appearance, while the follicular epithelium of thyroids from 1500 ppm males and females with slight hypertrophy had a low cuboidal appearance and the follicular epithelium of thyroids from 1500 ppm females with moderate hypertrophy had a high cuboidal appearance. Scattered foci of follicular cell hyperplasia were also noted in 2 out of 4 females at 1500 ppm, as compared to 0 out of 4 control females. The severity of this finding ranged from minimal to slight.

   Conclusion: Picolinafen caused slight anaemia and adverse changes in thyroid suggesting hyperthyroidism in dogs exposed for one year, however only at top dose of 42.7/47.1 mg/kg bw/d, well above a guidance value of 25 mg/kg bw/d for STOT RE 2 for one year exposure. The next lower dose level 4.4/5.2 mg/kg bw/d did not induce adverse effects in blood and thyroid, but the dose was 10 times

lower than the higher dose level. The study does not provide evidence meeting classification criteria of STOT RE 2.

5. In a 24-month dietary toxicity and oncogenicity study in rats (Anonymous 18, 1999; OECD TG 405, GLP), picolinafen was given in a diet to four groups of each 65 male and 65 female Sprague Dawley rats at dietary concentrations of 0, 50, 250 or 500 ppm (equal to 2.4/3.0, 12.1/15.0 or 24.5/31.0 mg/kg bw/d, respectively for males and females) for at least 24 months.

   Survival rates for the control, 2.4/3.0, 12.1/15.0 and 24.5/31.0 mg/kg bw/d groups were 24%, 29%, 31% and 29% for males and respectively 42%, 43%, 33% and 35% for females. No treatment-related clinical signs of toxicity were observed during the 24-month study period.

   A slight haemolytic anaemia (reduction of Hb, HCT and RBC less than 10% in comparison with control values) was noted for males and females at doses 12.1/15.0 or 24.5/31.0 mg/kg bw/d after 3 and 6 months, but not after 12 months of exposure. These effects were corroborated by changes in the spleen, i.e., a slight increase in the amount/severity of haemosiderin in males and females in the 12.1/15.0 or 24.5/31.0 mg/kg bw/d groups at 12 and 24 months, and an increase in absolute and relative spleen weights of males and females treated with 24.5/31.0 mg/kg bw/d at 12 and/or 24 months. No consistent statistically or biologically significant decreases in haematological parameters were observed for males or females at any dietary concentration tested at 18 and 24 months. Circulating white blood cells were comparable for all groups at all time points evaluated. No adverse effects in thyroid in any exposed group was reported.

   Conclusion: Picolinafen caused slight anaemia (Hb reduction below 10%) at dose levels 12.1/15.0 or 24.5/31.0 mg/kg bw/d only during first 6 months of exposure. The intensity of effects at dose levels equal to or above a guidance values of for STOT RE 2 for 12 and 24 months of exposure (25 and 12.5 mg/kg bw/d, respectively) were not severe enough to meet classification criteria for STOT RE 2.

6. In an 18-month dietary toxicity and oncogenicity study in mice (Anonymous 17, 1999; OECD TG 451, GLP), picolinafen was given in a diet to four groups of each 65 male and 65 female CD-1 albino mice at dietary concentrations of 0, 40, 400 or 800 ppm (equal to 0, 6.9/8.2, 68.6/81.0, 137.1/165.8 mg/kg bw/d, respectively for males and females) for 18 months.

   Survival was not affected by treatment with picolinafen. No treatment-related clinical signs of toxicity were observed during the 18-month study period. Similarly, no treatment-related effects on food consumption, mean body weight or overall body weight gain were noted in this study at dietary concentrations up to and including 800 ppm.

   No effect on haemoglobin concentration or RBC count was found in any exposed group during entire study, although percentage of reticulocytes in blood and mean corpuscular haemoglobin concentration in erythrocytes (MCHC) were increased at dose levels of 400 and 800 ppm, but only after first 3 months of exposure. In the

spleen statistically significant increased incidences of extramedullary haematopoiesis were noted for males and females at 800 ppm and a slight, not statistically significant increase was noted for males at 400 ppm too. Statistically significant increased incidences of haemosiderin deposition were noted for males and females at 800 ppm, and a slight, not statistically significant increase in the incidence of haemosiderin deposition was noted for females at 81.0 mg/kg bw/d, too. There was no increase in the severity of either of these findings at dose levels of 68.6/81.0 and 137.1/165.8 mg/kg bw/d compared to controls.

Conclusion: The results indicate that picolinafen may cause mild haemolytic anaemia in mice at dose levels of 68.6/81.0 (400 ppm) and 137.1/165.8 mg/kg bw/d (800 ppm). This is occurring only at doses above a guidance value of 16.6 mg/kg bw/d for STOT RE 2 for 18-month exposure. The observed effects do not meet classification criteria for STOT RE.

7. In a two-generation reproductive study in rats (Anonymous 21, 1999; OECD TG 416, GLP), picolinafen was given in a diet at concentrations of 0, 50, 250 or 500 ppm (equal to 3.7/4.2, 18/22 and 39/44 mg/kg bw/d, respectively for males and females in P-generation and the two highest doses were estimated to be for F-generation animals 17/27 and 34/55 mg/kg bw/d (m/f)).

   The P-generation, consisting of 30 male and 30 female rats per group, was treated for 10 weeks prior to a 14-day mating period to produce F1-litters. Weaned F1-offspring were selected to become the F1-parental generation which consisted of 30 male and 30 female rats per group. The F1-parental generation was treated for 10 weeks prior to a 14-day mating period to produce F2-litters. Both parental generations were treated during the 14-day mating periods as well as during the post-mating period. Mated females continued to be treated during the ensuing gestation, lactation and post-weaning periods.

   No treatment-related mortality or clinical signs of toxicity were observed for either the P- or F1-parental animals throughout the study period. Haematology evaluations were performed for all P- and F1-parental animals prior to scheduled sacrifice. Haemoglobin level in blood was reduced in males and females of P-generation by 7/8% (m/f) at dose of 250 ppm and by 9/13% (m/f) at dose of 500 ppm. The increased incidence of extramedullary haematopoiesis and brown pigmentation in reticuloendothelial cells was found in spleen in males and females of P-generation at dose levels of 250 and 500 ppm. In F1-generation haemoglobin level in blood was reduced in males and females by 3/7% (m/f) at dose of 250 ppm and by 7/13% (m/f) at dose of 500 ppm. The increased incidence of extramedullary haematopoiesis and brown pigmentation in reticuloendothelial cells was found in spleen in males and females of F1-generation at dose levels of 250 and 500 ppm.

   Conclusion: The reduction in haemoglobin concentration above 10% in females exposed for approximately 18 weeks in P- and F1-generation at 500 ppm (39/44 and 34/55 mg/kg bw/d) accompanied by increased incidence extramedullary haematopoiesis and severity of haemosiderosis in spleen and occurring below guidance value of 72.2 mg/kg bw/d (100 mg/kg bw/d x 13/18) is considered as an

adverse effect warranting classification STOT RE 2 (blood system).

28-day and developmental toxicity studies

Oral exposure

1. In a 28-day oral study in rats (Anonymous 15, 1993; OECD TG 407, GLP), picolinafen was given in a diet to five groups of each 10 male and 10 female Sprague Dawley (Crl:CD(SD)BR) rats at dietary concentrations of 25, 50, 100, or 1000 ppm for 28 days (resulting in dose level of 2.7/3.0, 5.4/5.9, 10.5/11.7 or 107/119 mg/kg bw/d, respectively for males and females).

   Haematological evaluations done at study termination revealed a reduction of haemoglobin by 9.1% and RBC by 11.4% in males and by 12.3% and 18% in females only at a dose level of 1000 ppm. There was a considerable increase in relative weight of spleen (by 83% in males, by 79% in females). There was also an increase in incidence of moderate or severe extra-medullary haematopoiesis and haemosiderin deposition in the spleen of males and females at 1000 ppm compared to controls.

   Conclusion: The reduction in haemoglobin concentration above 10% in females exposed for 28 days at 1000 ppm (119 mg/kg bw/d) accompanied by increased intensity extramedullary haematopoiesis and severity of haemosiderosis in spleen, occurring below guidance value of 300 mg/kg bw/d (100 mg/kg bw/d x 3) is considered as an adverse effect warranting classification STOT RE 2 (blood system).

2. In a 28-day oral study in mice (Anonymous 14, 1998; OECD TG 407, GLP), picolinafen was given in a diet to six groups of each 5 male and 5 female CD-1 albino mice at dietary concentrations of 0, 100, 1000, 2000, 3500 or 7000 ppm for 28 days (resulting in dose level of 23/28, 227/235, 438/598, 864/1140 or 1721/2019 mg/kg bw/d, respectively for males and females).

   There were no mortalities during the 28-day study period. The only clinical sign of toxicity noted was discoloured (pale) extremities for 8 out of 10 animals at 3500 ppm and 10 out of 10 animals at 7000 ppm. At termination of the study following was recorded: a slight, not statistically significant decrease in RBC for females at 7000 ppm compared to controls; a slight increase in reticulocytes for both sexes at 3500 ppm and for males (statistically significant) and females (not statistically significant) at 7000 ppm compared to controls; and statistically significant increases in Heinz body formation indicating oxidative damage to the haemoglobin in red blood cells for females at 3500 ppm and for males and females at 7000 ppm compared to controls. Haemoglobin concentration in blood was not reported. In microscopic investigation pigment deposition was noted in liver Kupffer cells for both sex starting from 2000 ppm and also in the spleen brown pigment deposition was noted together with extramedullary haematopoiesis for both sexes starting from 1000 ppm.

   Conclusion: The results indicate that picolinafen is causing a haemolytic anaemia in mice starting from the dose of 227/235 mg/kg bw/d (m/f) (1000 ppm) after 28

days of exposure, but lack of information on the haemoglobin level does not allow comparison of severity of the observed effects with classification criteria for STOT RE 2.

3. In a 28-day oral study in dogs (Anonymous 11, 1998; no OECD TG available, GLP) picolinafen was given to five groups of 2 male and 2 female Beagle dogs at dietary concentrations of 0, 100, 1000, 2000, or 10000 ppm for 28 days (resulting in dose level of 3.9/5.1, 48/44, 90/72 or 313/249 mg/kg bw/d respectively for males and females).

   There were no mortalities during the 28-day study period. A slight, haemolytic anaemia was noted at study termination for males and females in the 10000 ppm group. This anaemia was characterised by decreased haemoglobin, HCT, and RBC, as compared to concurrent controls, but numerical data were not provided. Additionally, increased reticulocyte counts were noted for one female at 2000 ppm and for both females at 10000 ppm.

   Absolute and relative thyroid/parathyroid weights were elevated for both sexes at 1000, 2000 and 10000 ppm, as compared to controls. Microscopically, the diffuse hyperplasia and hypertrophy were observed for all males and females at these same dietary concentrations. Hyperplasia was diagnosed as severe for animals at 2000 and 10000 ppm and mild-to-severe for animals at 1000 ppm. Hypertrophy of the thyroid gland was characterised by an increase in the height of the epithelium from low cuboidal to high cuboidal to columnar epithelium. Males at 1000 ppm as well as males and females at 2000 and 10000 ppm exhibited columnar epithelium, while females at 1000 ppm exhibited high cuboidal epithelium. Both females and one male in the control group exhibited low cuboidal epithelium and one male in the control group exhibited high cuboidal epithelium.

   Conclusion: The level of exposure was in the range of GVs of 30 – 300 mg/kg bw/d for STOT RE 2, but the data on haemolytic anaemia were not described in sufficient detail (no data on Hb and RBC) to allow a comparison with the classification criteria. However, the thyroid changes (a dose-dependent increase in weight and follicular cells hypertrophy and hyperplasia as observed in males and females at 1000, 2000 and 10000 ppm equalling to 48/44, 90/72 or 313/249 mg/kg bw/d (m/f)) are considered adverse effects justifying classification. Taking into account that mean absolute and relative thyroid/parathyroid weights in the male and female dogs at 48/44, 90/72 or 313/249 mg/kg bw/d (m/f) were more than two times larger than in the control animals, it is considered that this effect is adverse and warrants classification as STOT RE 2 (thyroid).

Dermal exposure

4. A 28-day dermal study in rats (Anonymous 8, 1999; OECD TG 410, GLP) was conducted in two phases. In the first phase, picolinafen was administered dermally to three groups of Sprague-Dawley rats (10/sex/group) at dose levels of 100, 200 or 1000 mg/kg bw/d. In the second phase, picolinafen was administered dermally to three groups of Sprague-Dawley rats (10/sex/group) at dose levels of 25, 50 or 75 mg/kg bw/d.

No mortalities, treatment-related clinical signs of toxicity or treatment-related signs of dermal irritation were noted in the first and second phase of the study.

Haematological evaluations done at day 27 of exposure revealed a reduction of haemoglobin by 13.5% and 14% in males and by 10.2% and 16.6% in females at dose levels of 200 or 1000 mg/kg bw/d, respectively. The decreases of Hb concentration in blood in animals exposed at 100 mg/kg bw/d and lower doses were less than 10%. Significant increases in absolute and/or relative (to body weight) spleen and liver weights were noted for males and females at 100, 200 and 1000 mg/kg bw/d compared to controls. Microscopically, extramedullary haematopoiesis and haemosiderin deposition in the spleen were noted for the majority of animals in the control, 100, 200 and 1000 mg/kg bw/d groups sacrificed at study termination (study day 27). However, the severity of extramedullary haematopoiesis and haemosiderin deposition was increased for males and females at 100, 200 and 1000 mg/kg bw/d compared to controls. Traces of extramedullary haematopoiesis and pigment deposition were diagnosed in the majority of animals in the control group and the same as mild-scored in the majority of animals at 100, 200 and 1000 mg/kg bw/d meaning that extramedullary haematopoiesis and pigment deposition were more severe in treated animals.

Conclusion: The level of exposure was in the range of GVs of 60 < C ≤ 600 mg/kg bw/d for STOT RE 2. The reduction above 10% in haemoglobin concentration in females and males exposed for 28 days at the dose level of 200 mg/kg bw/d accompanied by increased intensity of extramedullary haematopoiesis and severity of haemosiderosis in spleen, occurring below guidance value of 600 mg/kg bw/d is considered an adverse effect warranting classification for STOT RE 2 (blood system).

Oral exposure – Developmental

5. The developmental toxicity study in rats (Anonymous 20, 1999; OECD TG 414, GLP) with picolinafen was conducted in two phases. In the first phase, picolinafen was administered by oral gavage to three groups of mated female Sprague-Dawley rats (25 females/group) once daily from days 6 through 19 of gestation (14 days) at dose levels of 100, 500 or 1000 mg/kg bw/d. In the second phase picolinafen was administered by oral gavage to three groups of mated female Sprague-Dawley rats (17 females/group) once daily from days 6 to 19 of gestation (14 days) at dose levels of 5, 25 or 50 mg/kg bw/d.

   A reduction of haemoglobin concentration and HCT in blood of female rats exposed for 14 days at oral doses of 1000 mg/kg bw/d and lower were below 10% of the control values. There was a slight dose-dependent increase in intensity of extramedullary haematopoiesis and severity of haemosiderosis in spleen.

   Conclusion: The level of exposure was in the range of GVs of 60 < C ≤ 600 mg/kg bw/d for STOT RE 2 for exposure duration of 15 days. The results indicate that picolinafen causes mild haemolytic anaemia in pregnant rats at dose levels of 100-500 mg/kg bw/d, which is not meeting classification criteria for STOT RE 2.

6. In the developmental toxicity study in rabbits (Anonymous 19, 1998; OECD TG 414, GLP) picolinafen was administered by oral gavage to three groups of mated female New Zealand White rabbits (25 females/group) once daily from days 6 to 28 of gestation (23 days) at dose levels of 5, 20 or 50 mg/kg bw/d.

   A reduction of haemoglobin concentration by 14%, HCT by 15% and RBC by 27% in blood of female rabbits exposed for 23 days at oral dose of 50 mg/kg bw/d was associated with increased incidence and severity of deposition of haemosiderin in spleen.

   Conclusion: The level of exposure was in the range of GVs of 40 < C ≤ 400 mg/kg bw/d for STOT RE 2 for exposure duration of 23 days. Reduction of haemoglobin level in blood above 10% at dose of 50 mg/kg bw/d associated reduced HCT, RBC and with an increase in incidence and in severity of haemosiderin deposition in the spleen with increased level of exposure are regarded as adverse effect on erythrocytes meeting classification criteria and warranting STOT RE 2 (blood system).

Overall conclusion for STOT RE

RAC is of the opinion that

- reduction of Hb concentration in blood above 10% combined with marked increase of haemosiderosis in the spleen and liver and with increased intensity of medullary and extramedullary haematopoiesis observed in 90-day and 28-day repeated dose toxicity studies (Anonymous 10, Anonymous 15, Anonymous 8) and in 2-generation study in rats (Anonymous 21) as well as in developmental toxicity study in rabbits (Anonymous 19), and
- hypertrophy and hyperplasia of thyroid with large increase (over 2-fold) in thyroid weight in 28-day and 90-day repeated-dose toxicity studies in dogs (Anonymous 11, Anonymous 12)

warrant classification of picolinafen as **STOT RE 2; H373**: May cause damage to organs (blood system, thyroid) through prolonged or repeated exposure.

10.13 Aspiration hazard

No data available.

10.13.1 Short summary and overall relevance of the provided information on aspiration hazard

Criteria not applicable to solids according to Annex 3.10.1.6.2.a

10.13.2 Comparison with the CLP criteria

Criteria not applicable to solids according to Annex 3.10.1.6.2.a

10.13.3 Conclusion on classification and labelling for aspiration hazard

Criteria not applicable to solids according to Annex 3.10.1.6.2.a

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 36: Summary of relevant information on rapid degradability

Method	Test substance (purity)	Results	Remarks	Reference
Aqueous hydrolysis at pH 5, 7 and 9 OECD Guidelines No.111	picolinafen (98.7 %)	hydrolytically stable	--	Schlüter, H.(1997) CFS1997-034
Photodegradation in sterile water at pH 7 OECD 316	[^{14}C-pyridine]picolinafen (99.8 %) and [^{14}C-fluoroaniline]picolinafen (99.8 %)	DT_{50} = 54 – 88.8 d	--	McLaughin, S.P. (2012) 2011/1018566
Aqueous Photolysis of ^{14}C-AC 900001 SETAC Guideline Part 1, Section 10	^{14}C-picolinafen chem. purity > 96 %)	-	The study was replaced by study McLaughin (2012) due to erroneous integration of the background radioactivity on the TLC plates. Therefore, the study is not presented below.	Schlüter, H. (1998) CFS 1997-139; LUF 2000-4
Determination of the Direct Phototransformation in Buffered Medium at pH 7 OECD Draft Test Guideline: "Phototransformation of Chemicals in Water" (1992); BBA guideline Part IV, 6-1	non-labelled picolinafen (98.7 %)	Quantum yield of direct phototransformation in water at wavelength > 290 nm Φ: $2.14 \cdot 10^{-6}$ mol Einstein^{-1}	--	Knoch, E. and Yan, Z. (1998) ENV 97-028; LUF 2000-187
CL 153815: Aqueous Photolysis SETAC Guideline Part 1, Section 10	[^{14}C]-CL 153815 labelled at the 2 and 6 position of the pyridine ring (radiochemical purity 99 %, chemical purity 95.5 %)	Degradation of CL 153815 via photolysis is insignificant under natural conditions	--	Shah, J.F. and An,D. (1998) ENV 97-033; LUF 2000-5
Ready biodegradation	picolinafen (99.5 %)	Not readily biodegradable (7 % after 28 days)	--	Leberts, H. (1996) CFS 1996-039

OECD 301D				
Biodegradation in water/sediment systems SETAC Guideline, Part 1, Section 8.2.; OECD Draft Proposal	^{14}C-labelled picolinafen (99.5 %) and ^{14}C-CL 153815, radiolabel at 2,6-positions of the pyridine ring (99.0 %)	DT_{50} = 5.34-5.36 d (whole system) DT_{50} = 1.89 – 4.02 d (water)	SFO/Level P-I (new calculated by Mamouni & Jarvis 2012)	Yan, Z. (1999) ENV 98-019 and Mamouni, A. & Jarvis, T. (2012) DoclD 2012/1206414 for kinetic evaluation

11.1.1 Ready biodegradability

Author: Leberts, H.

Title: Study on the ready biodegradability of AC 900001, technical product

Date: 15/04/1996

Doc ID: Document No. CFS 1996-039, Study No. IF-96/04723-00

Guidelines: OECD 301D (Closed bottle test)

Deviations None

GLP: Yes

Acceptability: Yes

Material and methods

Technical grade picolinafen (purity 99.5 %) was incubated in a mineral nutrient solution with a composite inoculum consisting of a mixture of secondary effluent from a local municipal sewage plant (Taunusstein-Bleidenstadt) and aqueous extracts of a mixed soil at temperatures between 18.9 and 22.7 °C. Sodium benzoate was used as control substance. Immediately after mixing, and after 2, 7, 14, 21 and 28 days, the O_2 content in the vessels containing the test and control substance was measured using an O_2 probe. The biodegradability was calculated from the BOD (biological oxygen demand, the difference in O_2 content immediately after mixing and at the time of sampling) and the theoretical oxygen demand.

Results

The BOD of the test substance increased slightly from 0.11 mg BOD/L after 2 days to 0.21 mg BOD/L after 28 days. The values for the control substance were 2.81 and 3.80 mg BOD/l, respectively. The biodegradability of picolinafen was calculated to be 7 % after 28 days which is below the 60 % threshold value for ready biodegradability. The biodegradability of the control substance was 67 % after 7 days, and 76 % after 28 days.

Conclusion

Picolinafen has to be considered as not readily biodegradable.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

Author:	Schlüter, H.
Title:	Hydrolysis of ^{14}C-AC 900001
Date:	12/10/1997
Doc ID:	Report No. CFS 1997-034; WAS 2000-8
Guidelines:	OECD Guidelines, Volume 1, No. 111
Deviations	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The hydrolysis of picolinafen was investigated in sterile buffer solutions at pH values of 4, 7, and 9 using ^{14}C-picolinafen uniformly labeled in the aniline ring (chemical purity > 96 %, radiochemical purity 98 %; picolinafen purity 98.7 %). Triplicate samples at a concentration of approximately 0.02 µg/mL were kept in the dark for 5 days at 50 ± 0.1 °C. Following partition into dichloromethane the samples were analysed by radio TLC and HPLC at 0-time and 5 days after dosing.

Results

The recovery of radioactivity ranged from 93.3 to 105.0 % of the applied radioactivity. Recovered radioactivity was almost exclusively found to be organosoluble. TLC and HPLC analysis revealed the presence of unchanged parent compound only. There was no degradation of the compound in pH 4, pH 7, and pH 9 buffers over 5 days at 50 °C. Analysis of samples at the initiation and termination of the test indicated that no significant change in pH (±0.1 pH units) had occurred, and that the systems were sterile.

Conclusion

Picolinafen is stable to hydrolysis at pH 4, 7 and 9.

11.1.4 Other convincing scientific evidence

No data available.

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

No data available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

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

Author:	Yan, Z.
Title:	AC 900001 and CL 153815: Aerobic-anaerobic Transformation in Water-sediment Systems
Date:	18/02/1999
Doc ID:	Report No. ENV 98-019
Guidelines:	SETAC Guideline, Part 1, Section 8.2.; OECD Draft Proposal
Deviations	None
GLP:	Yes
Acceptability:	Yes

together with Mamouni (2012) for kinetic evaluation

Author:	Mamouni, A. Jarvis, T.
Title:	Determination of rates of decline for picolinafen and its metabolite CL 153815 in laboratory degradation studies according to the guidance within the FOCUS Kinetics Guidance Document
Date:	08/01/2012
Doc ID:	FOCUS (2006)
Guidelines:	BASF DoclD 2012/1206414
GLP:	Not applicable
Acceptability	Partly acceptable

Material and methods

The degradation of picolinafen and of the carboxylic acid soil metabolite (CL 153815) in two water/sediment systems was investigated in a flow-through test system using ^{14}C-labelled picolinafen and ^{14}C-CL 153815 (radiolabel at 2,6-positions of the pyridine ring, radiochemical purity 99.5 % and 99.0 %, resp.). Separate sets of experiments were carried out for both test substances.

Characteristics of the two water/sediment systems used for this study are specified in table below. The river system was collected from North Dakota and had a coarse texture (sandy loam) with low organic carbon content (3.1 %). The pond system was collected from North Carolina and had a fine texture (loam) with high organic carbon content (5.2 %). Samples of each water and corresponding sediment were placed into 500 mL cylindrical glass bottles at a water : sediment ratio of 4:1. Moistened carbon dioxide-free air was passed through the water surface. After an acclimatisation period, the test substance ^{14}C-Picoliafen or ^{14}C-CL 153815 was applied onto the water surface at a dose rate of approximately 0.04 ppm (equivalent to an application rate of 400 g as/ha assuming distribution in a water depth of 100 cm). The incubation bottles were connected to two traps in series to collect carbon dioxide and organic volatiles. The temperature was maintained at 20 ± 1 °C throughout the study. Duplicate incubation units were removed for analysis at time intervals of 0, 1, 2, 3, 7, 14, 30, 62 and 100 days after application of the test substance. The water phase of each sample was separated from the sediment by centrifugation, and the amount of radioactivity in the water was measured directly by liquid scintillation counting (LSC). Sediments were exhaustively extracted with acetonitrile and partly with other organic solvents (acetone, methanol, methylene chloride). 0.5 N NaOH solutions were also used to further extract the residues remaining in the sediments. The water samples and sediment extracts were concentrated and then analysed by both high-performance liquid chromatography

(HPLC) and thin layer chromatography (TLC). The non-extractable radioactivity remaining in the sediments was analysed by combustion.

Additional water/sediment samples were fortified at a dose rate of 0.2 ppm with a mixture of ^{14}C-picolinafen and ^{15}N-picolinafen to facilitate the identification of degradation products by mass-spectrometry (LC/ESI/MS).

Table 37: Characteristics of the water/sediment systems with picolinafen and the acid metabolite (CL 153815)

	River	**Pond**
Water		
pH	8.1	6.8
Hardness (mg equivalent $CaCO_3$/l)	720	13
Total N (ppm)	8	5
Total P (ppm)	0.4	0.5
Sediment		
Characterisation (USDA classification)	Sandy loam	Loam
% Sand % Silt % Clay	73 14 13	51 34 15
% Organic matter (% Organic carbon)	5.2 (3.1)	8.5 (5.2)
pH_{Water}	7.8	5.2
pH_{KCl}	7.4	4.4
Cation exchange capacity (meq/100g)	27.4	8.3
1/3 Bar water holding capacity (%)	47.6	45.0
Microbial biomass at the beginning of the study (mg C/100 g sediment)	64.6	27.8
Microbial biomass at the end of study with picolinafen (mg C/100 g sediment)	66.3	70.9
Microbial biomass at the end of study with CL 153815 (mg C/100 g sediment)	61.2	65.1

Results

Picolinafen: The distribution of the radioactivity of the water/sediment studies spiked with picolinafen is summarised in table 38. Total recoveries ranged from 93.4 to 103.4 % and 93.5 to 103.6 % of the applied radioactivity in the river and pond water-sediment systems, respectively. 40.1 % of the radioactivity in the river system and 70.6 % in the pond water/sediment system was immediately removed to the sediment phase at the starting day 0 of the experiment. The remaining radioactivity gradually dissipated from the water to the sediment phase in both systems. After 100 days of incubation, 9.3 and 0.4 % AR remained in the water phase of the river and pond systems, respectively. The water/organic solvent extractable radioactivity in the water-sediment systems decreased over time with 36.8 and 16.9 % of the applied radioactivity being extracted at 100 days in the river and pond systems, respectively. The 0.5 N NaOH extractable residues (which represent the residues tightly bound to sediment organic matters and likely to be non-bioavailable) generally increased over time to 57.9 % AR at day 100 in the river system and to 31.0 % AR at day 62 in the pond system with a subsequent decrease to 18.2 % AR at day 100. The non-extractable residues in the river sediment increased to a maximum of ~13.0 % AR at day 7 and then fluctuated between 6.2 and 10.5 % AR throughout the study. The non-extractable residues in the pond sediment increased over time and reached ~ 64.5 % AR at day 100. This strong binding of picolinafen-derived residues to the sediment was probably due to the high organic matter and silt/clay contents of the pond sediment. Only a small amount of $^{14}CO_2$ was detected in the NaOH traps accounting for ~ 2 % AR in both the river and the pond system. No significant amount of radioactivity (0.1 to 0.3 % AR) was found in the ethylene glycol traps in either of the test systems.

Table 38: Distribution of recovered radioactivity (% AR, mean of two replicates) of the water/sediment study with picolinafen

Days after application	Volatiles	Water layer	Sediment solvent extractable	Sediment NaOH extractable	Sediment non-extractable	Total
River system						
0	-	53.3	39.2	0.2	0.9	93.4
1	0	63.4	34.9	-	1.3	99.5
2	0	58.9	36.5	-	2.6	98.0
3	0	58.6	31.9	-	3.6	94.1
7	0	58.1	28.1	-	13.0	99.2
14	0.2	37.1	39.2	16.1	7.6	100.2
30	0.7	28.4	30.8	33.8	8.1	101.7
62	1.3	21.1	32.1	36.8	10.5	101.8
100	2.5	9.3	27.5	57.9	6.2	103.4
Pond system						
0	-	23.0	68.7	0	1.9	93.5
1	0	45.1	55.1	-	3.4	103.6
2	0	47.1	47.0	-	3.9	98.0
3	0.1	36.3	58.1	-	8.4	102.9
7	0.2	33.8	34.6	0.4	29.2	98.1
14	0.4	19.1	41.5	11.9	26.1	98.9
30	1.1	14.6	34.6	17.1	32.3	99.6
62	1.8	1.4	20.2	31.0	41.4	95.7
100	2.5	0.4	16.5	18.2	64.5	102.2

The distribution of picolinafen and the metabolite CL 153815 determined by HPLC is presented in table 39, the distribution of picolinafen and the metabolite CL 153815 determined by TLC is presented in table 40.

A large portion of picolinafen dissipated already on day 0 into the sediment phase. Picolinafen then degraded quickly both in the water as well as in the sediment phase to form CL 153815 in both water sediment systems. In the river system, no active substance was detected anymore at the end of the study (day 100), in the Pond system only 1.9 %/ 2.7 % AR (HPLC/ TLC) was still measured at the study end. Metabolite concentrations of CL 153815 reached maxima of 92.4 %/ 94.5 % AR (HPLC/ TLC) in the river system on day 100 and 49.2 % AR on day 62 (HPLC) and 53.6 % AR on day 30 (TLC) in the pond system. . Maximum amounts of 41.3 %/ 38.9 % AR (HPLC/ TLC) and 31.5 %/ 31.4 % AR HPLC/ TLC) were measured on day 7 in the water phase with subsequent decline. . In the sediment, maximum concentrations of CL 153815 were observed at the end of the study (day 100) with 83.1 %/85.3 % AR (HPLC/TLC) in the river system and at day 62 with 47.9 %/ 46.9 % (HPLC/TLC) in the pond system. The distribution of the metabolite suggests that it was mainly formed in the water phase but was transferred to the sediment layer afterwards.

Table 39: Degradation of picolinafen and formation of CL 153815 in water-sediment systems (% AR, mean of two replicates) determined by HPLC

Days after	Water Layer		Sediment Layer		Total System	
application	picolinafen	CL 153815	picolinafen	CL 153815*	picolinafen	CL 153815*
River System						
0	52.2	1.0	39.0	0.1	91.2	1.1
1	52.1	11.3	34.8	0.0	86.9	11.3
2	43.2	15.6	36.5	0.0	79.7	15.6
3	33.3	25.3	31.6	0.1	64.9	25.3
7	16.8	41.3	26.3	1.8	43.1	43.1
14	0.3	36.8	0.0	55.2	0.3	92.0
30	0.0	28.4	1.8	61.7	1.8	90.0
62	0.0	21.0	0.2	68.6	0.3	89.6
100	0.0	9.3	0.0	83.1	0.0	92.4
Pond System						
0	22.7	0.3	68.6	0.0	91.3	0.3
1	37.4	7.6	55.0	0.1	92.3	7.7
2	26.0	21.0	46.6	0.3	72.6	21.3
3	19.6	16.7	56.1	1.9	75.7	18.6
7	2.3	31.5	24.6	10.0	26.8	41.5
14	0.4	18.6	22.5	30.3	22.9	48.9
30	0	14.6	10.3	41.0	10.3	55.5
62	0	1.4	2.4	47.9	2.4	49.2
100	0	0.0	1.9	32.2	1.9	32.2

*including NaOH extract

Table 40: Degradation of picolinafen and formation of CL 153815 in water-sediment systems (% AR, mean of two replicates) determined by TLC

Days after	Water Layer		Sediment Layer		Total System	
application	picolinafen	CL 153815	picolinafen	CL 153815*	picolinafen	CL 153815*
River System						
0	53.3	0.0	38.3	0.0	91.6	0.0
1	51.3	11.3	34.4	0.0	85.7	11.3
2	43.3	14.7	35.7	0.0	79.0	14.7
3	31.3	27.2	31.8	0.0	63.1	27.2
7	15.0	38.9	26.0	1.4	40.9	40.3
14	0.1	36.6	0.2	53.7	0.3	90.4
30	0.0	28.3	3.7	60.4	3.7	88.7
62	0.0	20.9	1.2	67.6	1.2	88.5
100	0.0	9.2	0.0	85.3	0.0	94.5
Pond System						
0	23.0	0.0	67.3	0.0	90.3	0.0
1	38.0	7.1	54.6	0.0	92.6	7.1
2	27.2	19.8	46.0	0.0	73.1	19.8
3	19.0	17.2	55.2	2.0	74.3	19.2
7	2.3	31.4	26.1	8.3	28.4	39.6
14	0.3	18.6	23.3	29.3	23.6	47.8
30	0	14.3	11.0	39.2	11.0	53.6
62	0	1.3	3.0	46.9	3.0	48.2
100	0	0.0	2.7	31.7	2.7	31.7

*including NaOH extract

The degradation kinetics was evaluated using the modeling program CAKE v 1.3. Single first-order (SFO), first-order multi-compartment (FOMC or Gustafson-Holden) and bi-exponential (DFOP) models were

applied to simulate the kinetic of picolinafen and of metabolite CL 153815, where they were applied directly to the system. For simulating CL 153815 in the water/sediment study spiked with picolinafen, a SFO model was applied to simulate the metabolite.

Table 41: Kinetic evaluation of the dissipation of picolinafen from the water phase of the water/ sediment systems river and pond

Water/ Sediment system	Kinetic Model	Parameter	Value	σ	p-value (t-test)	error χ^2 test (%)	DT_{50} (d)	DT_{90} (d)
River	SFO	M_0	56.91	2.512	4.135E-08	10.27	4.02	13.35
		K	0.1725	0.02089	3.726E-05			
Pond	SFO	M_0	54.44	2.29	1.817E-07	6.36	1.89	6.29
		K	0.366	0.02329	2.102E-06			

Table 42: Kinetic evaluation of the degradation of picolinafen and CL 153815 in the total system of the water/ sediment systems river and pond

Water/ Sediment system	Kinetic Model	Compart-ment	Parameter	Value	σ	p-value (t-test)	error χ^2 test (%)	DT50 (d)	DT90 (d)
River	SFO	Parent	M_0	96.28	4.646	3.323E-12	11.58	5.36	17.79
			k_parent	0.1295	0.01658	9.094E-07			
		Met	ff_met	0.9626	0.1045	1.277E-05	10.14	578	19198
			k_met	0.00012	0.01409	*0.4667*			
Pond	SFO	Parent	M_0	96.75	5.343	1.817E-11	13.09	5.34	17.74
			k_parent	0.1298	0.01609	6.202E-07			
		Met	ff_met	0.6876	0.07132	7.329E-08	7.70	96.03	319
			k_met	0.007218	0.001508	1.452E-04			

The resulting DT_{50} and DT_{90} values describing the dissipation of picolinafen from the water phase and the degradation in the total system of both water/sediment systems are summarised in table below.

Table 43: DT_{50} and DT_{90} values for the dissipation of picolinafen from the water phase and the degradation in the total system of the water/sediment systems river and pond

Water/ Sediment system	Dissipation Water phase (Persistence endpoint)			Degradation total system (Persistence and modelling endpoint)		
	DT_{50}	DT_{90}	Kinetic/ Fit	DT_{50}	DT_{90}	Kinetic
River	4.02	13.35	SFO/ chi^2: 10.27 %	5.36	17.79	SFO/ chi^2: 11.58 %
Pond	1.89	6.29	SFO/ chi^2: 6.36 %	5.34	17.74	SFO/ chi^2: 13.09 %

Conclusion

SFO kinetics gave good visual and statistically reliable fit for the dissipation of picolinafen from the water phase and for the degradation in the total system for both water sediment systems. Dissipation rates for the water phase for all systems (river, pond) are acceptable. DT_{50} were 5.34 and 5.36 days (DT_{90} 17.74 and 17.79 days).

11.1.4.4 Photochemical degradation

Author:	McLaughin, S.P. Lian, P.
Title:	Photodegradation of picolinafen in water, based on the OECD 316 – Direct photolysis Guideline, Tier I and II
Date:	08/02/2012
Doc ID:	BASF DocID 2011/1018566
Guidelines:	OECD 316 (Oct 2008)
Deviations	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

[^{14}C-pyridine]picolinafen and [^{14}C-Fluoroaniline]picolinafen was initially prepared as solutions in acetonitrile.

For Tier I testing was performed at a concentration of 10 µg/mL in sterile aqueous buffer solution (pH 7, 0.01 M sodium phosphate) and acetonitrile at a 1:1 ratio. For ultraviolet/visible spectral analysis, the picolinafen test solution was scanned from 290 to 800 nm using a UV/ VIS spectrophotometer.

For Tier II testing, 5 mL samples of sterile aqueous buffer (pH 7, 0.01 M sodium phosphate) were treated separately with 50 µL of stock solution of each label at a concentration of approximately 18 µg/L. Sterile quartz sample tubes (100 mm length by 12 mm diameter), equipped with Teflon®-lined caps were used for irradiated samples. For the dark controls, sterile pyrex sample tubes with Teflon®-lined silicon septum screw caps were used. Temperature was maintained at 25 ± 2 °C. Test samples were continuously irradiated with artificial light from a Xenon arc lamp using a wavelength from 290 to 380 nm. The emission spectrum of the Xenon arc lamp showed a good overlap with the spectrum of natural sunlight obtained in Massachusetts at 42 N and 70°W taken in June 2008 at 13h. The photolysis cells and dark control cells were fitted with traps for CO_2 and volatile compounds. The trapping solutions used were NaOH for CO_2 and ethylene glycol for volatile organic compounds.

Duplicate irradiated and dark control samples were analysed immediately after the test substance was placed into the test vessels (day 0) and after 2, 4, 7, 10 and 15 days of irradiation. Dark control samples were analysed after 7 and 15 days of incubation. The sterility of the prepared buffer and dosed samples and the pH of each sample were confirmed at the start and end of the study.

At each sampling interval, the volume in each test tube was measured. The samples were analysed by LSC and reverse phase HPLC. The limit of quantification of the HPLC was calculated to be at least 1 % of the applied radioactivity. Selected samples were analysed by TLC.

Results

The rate constant of picolinafen at pH 7 determined during the Tier I testing was 1703.81 day $^{-1}$. Estimated half lifes of picolinafen were ≤ 30 days at 30, 40 and 50 N, thus Tier II testing was also performed.

The overall recovery of radioactivity for [^{14}C-pyridine]picolinafen in the Tier II testing ranged from 94.2 to 101.6 % of the applied radioactivity in the irradiated samples and from 94.8 – 100 % in the dark controls. For [^{14}C-fluoroaniline]picolinafen, the overall recovery of radioactivity ranged from 92.9 to 99.1 % of the applied radioactivity in the irradiated samples and from 94.2 – 99.0 % in the dark controls. Approximately 17.5 % and 10.3 % of the radioactivity associated with [^{14}C-pyridine]picolinafen and [^{14}C-

fluoroaniline]picolinafen was degraded after 15 days of irradiation. No degradation products >5 % were observed in the irradiated samples. Under dark conditions, picolinafen was stable.

Experimental DT_{50} values for picolinafen were determined with the modelling programme CAKE using SFO kinetics and are presented in table below. The mean half-life of 76.4 days is equivalent to approximately 217 days of summer natural sunlight at 40° latitude.

Table 44: 1st order rate constants and half-lives for the direct photolysis of with [^{14}C-pyridine]picolinafen and [^{14}C-fluoroaniline]picolinafen at pH 7

Samples	**Rate constants ($days^{-1}$)**	**Chi^2 (%)**	**DT_{50} (days)**
[^{14}C-pyridine]picolinafen	0.01084	1.92	64.0
[^{14}C-fluoroaniline]picolinafen	0.007807	1.91	88.8

Conclusion

The study was performed according to guideline and is considered acceptable by the RMS. The study was performed by the Notifier to confirm, if the apparent photodegradation of picolinafen in the study Schlüter, 1998 was mainly due to an erroneous integration of the radioactivity of the TLC plates, which would have resulted in an overestimation of the picolinafen degradation. Since, the experimental DT_{50} values of picolinafen at pH 7 in this study are significantly longer (54 and 88.8 days) than the DT_{50} value (31.4 d) in the study Schlüter (1998), such an erroneous integration appears to have happened. Thus, the DT_{50} values of the new study should replace the study results of Schlüter, 1998.

It can be concluded that degradation of picolinafen via photolysis is insignificant under natural conditions.

Author: Knoch, E., Yan, Z.

Title: Picolinafen (AC 900001): Determination of the Direct Phototransformation in Buffered Medium at pH 7

Date: 02/11/1998

Doc ID: Report No. ENV 97-028; LUF 2000-187

Guidelines: OECD Draft Test Guideline: "Phototransformation of Chemicals in Water" (1992); BBA guideline Part IV, 6-1

Deviations None

GLP: Yes

Acceptability: Yes

Materials and methods

The quantum yield of picolinafen was investigated in sterile pH 7 buffer using non-labelled picolinafen (purity 98.7 %). The initial concentration of picolinafen in pH 7 buffer, with 0.1 % acetonitrile, was 0.05 µg/mL. Samples were exposed continuously to light from a xenon arc lamp, which simulated the spectrum of sunlight (Heraeus Suntest apparatus) at a temperature of 20 ± 2 °C. Control samples were maintained in the dark at 20 °C. Uranyl nitrate/oxalic acid actinometry was used to determine the number of incident photons. Sampling times were 0, 1, 6, 24, 48 and 72 hours. After addition of acetonitrile, the samples were analysed by HPLC. Molar absorption coefficients were calculated from the absorption spectrum at 2.5 nm increments over the wavelength range of 290 – 490 nm.

Results

The quantum yield of picolinafen was determined to be $2.14 \cdot 10^{-6}$. No photodegradation products were observed. Average recovered concentrations of picolinafen after 72 hours were 84.4 % and 96.6 % for the irradiated and the dark control samples, respectively. The DT_{50} using the artificial light source (relative intensity: 2.34 sun hours per instrument hour) was calculated to be 290.7 hours (12.1 days) under the laboratory conditions. This indicates that the test substance was slowly photolyzed under the test conditions.

Conclusion

Picolinafen was slowly degradated by photolysis. Therefore, no environmental half-life calculation was performed in this study.

Author:	Shah, J. F.; An, D.
Title:	CL 153815: Aqueous Photolysis
Date:	04/09/1998
Doc ID:	Report No. ENV 97-033; LUF 2000-5
Guidelines:	SETAC Guideline Part 1, Section 10
Deviations	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The photodegradation of CL 153815 (acid metabolite of picolinafen) in sterile pH 5, 7 and 9 buffer solutions was investigated using [^{14}C]-CL 153815 labelled at the 2 and 6 position of the pyridine ring (radiochemical purity 99 %, chemical purity 95.5 %). The concentration of the test substance used in the test systems was approximately 7 µg/mL. The dosed solutions were exposed to simulated sunlight from a xenon arc lamp which had been filtered to remove wavelengths less than 290 nm. The samples were irradiated continuously for 7 days at a temperature of 20 ± 3 °C. Control samples were maintained in the dark at 20 ± 3 °C. The light-exposed samples were assayed after 0, 26, 50, 74, 122 and 170 hours (164 hours for pH 7 samples) of irradiation. The dark samples were assayed at 0, 24, 48, 72, 120, and 168 hours after dosing. Aliquots of the samples were analysed by reversed-phase HPLC with radiochemical flow detector. A flow-through system was used for the pH 5 samples to collect organic volatiles and carbon dioxide.

Results

At pH 5 the test substance CL 153815 accounted for 99.7 % and 97.9 % of the total radioactivity after 26 and 170 hours of continuous irradiation, respectively. A minor degradate, which reached a maximum of 1.7 % of the initial applied radioactivity after 170 hours of irradiation, was identified as CL 170568 (6-hydroxy picolinic acid). There was insufficient degradation over the course of the study to allow for the calculation of a DT_{50}.

At pH 7 in the irradiated samples CL 153815 accounted for 98.6 % of the total radioactivity 164 hours of continuous exposure, respectively. There was insufficient degradation over the course of the study to allow for the calculation of a DT_{50}.

CL 153815 was stable to irradiation with simulated sunlight at pH 9 under the test conditions.

Dark control samples were stable throughout the course of the study.

Conclusion

Small amounts of CL 153815 were photolysed under neutral and acidic conditions; however, degradation was too small to derive DT_{50} values.

The study is considered acceptable by the RMS. It can be concluded that degradation of CL 153815 via photolysis is insignificant under natural conditions.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for this dossier.

11.3 Environmental fate and other relevant information

Not relevant for this dossier.

11.4 Bioaccumulation

Table 45: Summary of relevant information on bioaccumulation

Method	Test substance	Results	Remarks	Reference
OECD 305E (flow through)	[pyridine-2,6-^{14}C]-Picolinafen Purity: > 99 %	Kinetic bioconcentration factors for parent, whole fish were 420 and 730 for 2 ppb and 20 ppb test concentrations, respectively. Relevant: BCF_{kl} = 617	Reliability: 1	Anonymous 22, 1998
OECD 305E (flow through)	[p-fluoroanilineU-^{14}C]-Picolinafen Purity: > 98 %	Kinetic bioconcentration factors for parent, whole fish were 540 and 600 for 2 ppb and 20 ppb test concentrations, respectively Relevant: BCF_{ssl} = 561	Reliability: 1	Anonymous 22, 1998

11.4.1 Estimated bioaccumulation

The log $K_{o/w}$ of Picolinafen is 5.4 at 25°C. Therefore, there is an indication for bioaccumulation potential of Picolinafen.

11.4.2 Measured partition coefficient and bioaccumulation test data

Author: Anonymous

Title: CL 900001: Uptake, depuration, bioconcentration and Metabolism of Carbon-14 Labelled CL 900001 in Bluegill Sunfish (*Lepomis macrochirus*) under Flow-

	Through Conditions
Date:	1998
Doc ID:	MET 98-004 , WAT1999-519
Guidelines:	OECD 305E, EPA OPPTS 850.1730
GLP:	Yes
Validity:	Valid
Previous evaluation:	In initial DAR (2000)

Materials and methods

The test substance picolinafen was prepared for the study by isotopic dilution of [pyridine-2, 6-^{14}C]- AC 900001, [pyridine- 15N]- AC 900001, and non-radiolabelled AC 900001 to achieve a specific activity of 11.09 µCi/mg, and a radiochemical purity of >99 % and by isotopic dilution of [p-fluoroaniline-U-^{14}C]- AC 900001 and non-radiolabelled AC 900001 to achieve a specific activity of 10.63 µCi/mg, and a radiochemical purity of >98 %. The [15N] isotope was added as a mass marker to aid in mass spectrometric analysis of AC 900001-derived residues in the fish and water samples. The positions of the carbon-14 labels were considered to be metabolically stable to allow determination of the metabolic profile. The specific activities afforded nominal detection limits (NLD) of approximately 7 ppb for the fish fillet and 5 ppb for the fish viscera when 0.25 g of sample was analysed, and 0.2 ppb for water when 10 mL of water was analysed. The average minimum quantifiable limits (MQL) of 0.1 ppb for water and 4 ppb for fish fillet and viscera were determined by recovering a fortified amount of radioactivity from the various sample matrices.

The in-life phase of the AC 900001 bluegill sunfish study was conducted at ABC Laboratories, Inc., Columbia, MO. The bioconcentration study consisted of an uptake (exposure) phase of 28 days and a depuration phase of 14 days, during which both fish and water were sampled at periodic intervals. During the exposure period, a flow-through proportional diluter system was used to distribute and maintain the appropriate test substance concentrations in the aquarium water.

The test substance concentrations used for the study are safe treatment levels and represent less than 1/10 the acute toxicity for AC 900001 to bluegill sunfish [LC_{50} (96 hr) >0.57 mg/L].

Radioanalysis of fillet (edible) and viscera (inedible) portions was performed periodically during the exposure and depuration period. Total radioactive residues (TRR) were determined by direct liquid scintillation counting (LSC) of water samples and combustion of the fish tissues to yield $^{14}CO_2$ that was trapped by an amine and quantified by LSC.

In this study, the co-solvent used was dimethylformamide (DMF). The concentration of the test substance in the aquarium chambers was maintained within + 20 % of the mean of the measured values during the uptake phase. The temperature variation was less than + 2 %. The concentration of dissolved oxygen did not fall below 60 % saturation. The total organic carbon (TOC) ranged from 38 to 50 mg/L for all treatment groups during the acclimation and exposure periods. The TOC concentrations were attributed to the presence of the co-solvent at 0.1 mL/L in all treatment groups.

The bioconcentration factor (BCF) and TRR for AC 900001, expressed as ppb equivalents of [^{14}C]-AC 900001, in the water and fish samples and at the various time intervals, were calculated. The whole fish residues were calculated from the sum of the mean percent contribution of fillet and viscera to whole fish for each treatment group as measured on each sampling day of the study.

The aquarium water samples were extracted using BakerBond laminar C18 Speedisks. Fish fillet and viscera were extracted with methanol:acetonitrile:water (800:800:400, v/v/v). The post-extracted solids (PES) of fillet and viscera were digested with pepsin/0.1N HCl followed by hydrolysis of the residual PES with 6N HCl. The extracts of the aquarium water, fish fillet and viscera, and the enzyme digests and the acid hydrolysates of the PES were analysed by HPLC on a C18 reversed phase column using a gradient mobile

phase system followed by LSC to determine the radioprofiles and to quantitate the components of the radioactive residue in the aquarium water and the fish fillet and viscera.

AC 900001 and the metabolites were isolated from the fish viscera by HPLC and by solvent partitioning between hexane, methylene chloride, and water. AC 900001 and the metabolites were identified by liquid chromatography/mass spectrometry (LC/MS) and negative ion mass spectrometry (NIMS).

Results and Discussion

From the uptake and depuration data, the ^{14}C-AC 900001-derived radioactivity in the whole fish appeared to reach steady state (plateau) by day 14 of the uptake period after exposure to both pyridine-^{14}C-labelled and p-fluoroaniline-^{14}C-labelled AC 900001. The highest residues were observed in the viscera (1800 ppb and 2000 ppb for treatments at 2 ppb, and 31000 ppb and 20000 ppb for treatments at 20 ppb). The ^{14}C residue levels in whole fish were reduced to 1.2 % - 4.1 % of the steady-state concentrations 14 days after the start of depuration. The bioaccumulation parameters for the AC 900001-derived radioactivity (TRR) and for picolinafen for whole fish for each treatment group are summarized as follows:

Table 46: Bioaccumulation parameters for TRR (total radioactive residue)

	Treatment Group (Nominal Exposure Concentration)			
Parameter	**Group B (2 ppb)**	**Group C (20 ppb)**	**Group D (2 ppb)**	**Group E (20 ppb)**
Bioconcentration Factor (BCF)	370	470	380	500
K1, uptake rate constant (ppb fish/ppb water/Day)	190	200	290	240
K2, depuration rate constant (Day^{-1})	0.51	0.43	0.76	0.48
Time to 90 % steady-state, Days	4.5	5.4	3.0	4.8
Time for 50 % depuration, Days	1.4	1.6	0.92	1.5
Time for 95 % depuration, Days[a]	5.9	7.0	3.9	6.2

[a)] Hand calculated using BIOFAC data

Table 47: Bioaccumulation parameters for picolinafen (AC 900001)

	Treatment Group (Nominal Exposure Concentration)			
Parameter	**Group B (2 ppb)**	**Group C (20 ppb)**	**Group D (2 ppb)**	**Group E (20 ppb)**
Bioconcentration Factor (BCF)	420	730	540	600
K1, uptake rate constant (ppb fish/ppb water/Day)	170	420	420	300
K2, depuration rate constant (Day^{-1})	0.41	0.58	0.78	0.49
Time to 90 % steady-state, Days	5.6	4.0	2.9	4.7
Time for 50 % depuration, Days	1.7	1.2	0.89	1.4
Time for 95 % depuration, Days[a]	7.3	5.2	3.8	6.1

[a)] Hand calculated using BIOFAC data.

The BIOFAC BCF values were calculated using the parent AC 900001 water concentration to estimate the steady-state concentration of AC 900001 during the exposure period. Due to the lower concentration of

parent AC 900001 in water versus whole fish, the BIOFAC calculated TRR steady-state BCF (BCFss) numbers for the AC 900001-derived residues are lower than the equivalent BIOFAC calculated parent AC 900001 BCF numbers for the parent AC 900001 for the four treatment groups.

The lipid content of whole fish for day 28 of exposure was calculated from the sum of mean percent contribution of fillet and viscera to whole fish for each treatment group. The lipid content of bluegill sunfish was 4.5 %, 5.9 %, 6.4 % and 6.6 % for whole fish in treatment groups B, C, D, and E, respectively.

Table 48: Bioaccumulation parameters for picolinafen based on parent

	Treatment Group (Nominal Exposure Concentration)			
	Pyridine-2,6-^{14}C		**p-Fluoroaniline-U-^{14}C**	
Parameter	**(2 ppb)**	**(20 ppb)**	**(2 ppb)**	**(20 ppb)**
Bioconcentration Factor (BCF), steady state	530 (589*)	640	560 (438*)	740 (561*)
Time to Steady-State, Days	28	28	28	28
Bioconcentration Factor (BCF), kinetic	420	730 (617*)	540	600
K1, Uptake rate constant (ppb fish/ppb water/Day)	170	420	420	300
K2, Depuration rate constant (Day^{-1})	0.41	0.58	0.78	0.49
Time to Steady-State, Days	5.6	4	2.9	4.7
Time for 50 % depuration, Days	1.7	1.2	0.89	1.4

* lipid content normalised to 5 %

A BCF value of 617 of whole fish based on parent and normalized to 5 % lipid content was derived from this 28-d flow-through study on *Lepomis macrochirus*. Time for 50 % depuration is 1.2 d, and after 14 d depuration of picolinafen is > 95 %.

The study is considered valid and reliable. It is relevant for classification purposes.

11.5 Acute aquatic hazard

Table 49: Summary of relevant information on acute aquatic toxicity

Method	**Species**	**Test material**	**Results[1]**	**Remarks**	**Reference**
EPA Guideline 72-1(c), OECD Guideline 203, and EC Guideline C1	*Oncorhynchus mykiss*	Picolinafen (purity: 97.8 %)	LC_{50} (96 h) > 0.68 mg a.s./L (mean measured)	Reliability: 1	Anonymous 23 (1998) ECO 96-309
US EPA Guideline 72-1(a), OECD Guideline 203, and EC Guideline C1	*Lepomis macrochirus*	Picolinafen (purity: 97.8 %)	LC_{50} (96 h) > 0.57 mg a.s./L (mean measured)	Key study Reliability: 1	Anonymous 24 (1998) ECO 96-308
US EPA Guideline 72-1(c), OECD Guideline 203, and EC Guideline C1	*Oncorhynchus mykiss*	Metabolite CL 153815* (purity: 100 %)	LC_{50} (96 h) > 100 mg/L (mean measured)	Reliability: 1 Supplementary information	Anonymous 25 (1998) ECO 97-351
OECD 203 (1992); EC 440/2008 C.1	*Oncorhynchus mykiss*	Metabolite CL 7693* (purity:	LC_{50} (96 h) = 19.9 mg/L (mean measured)	Reliability: 1 Supplementary information	Anonymous 26 (2011) 61323230

		99.7 %)			
US EPA Guideline 72-2, OECD 202 Part A, and EC Guideline C2	*Daphnia magna*	Picolinafen (purity: 98.7 %)	EC_{50} (48 h) > 0.45 mg a.s./L (mean measured)	Reliability: 1	Wisk (1998) ECO 96-182
US EPA Guideline 72-2, OECD 202 Part A, and EC Guideline C2	*Daphnia magna*	Metabolite CL 153815* (purity: 100 %)	EC_{50} (48 h) > 98 mgL (mean measured)	Reliability: 1 Supplementary information	Drottar et al. (1998) ECO 97-352
OECD 202 (2004); EC 440/2008 C.2	*Daphnia magna*	Metabolite CL 7693* (purity: 99.7 %)	EC_{50} (48 h) = 0.254 mg/L (mean measured)	Reliability: 1 Higher toxicity than parent	Kley & Deierling (2011) 61322220
OECD 201 and EC Guideline C3	*Pseudokirchneriella subcapitata*	Picolinafen (^{14}C-labeled) (purity: 97.8 %)	E_rC_{50} (72 h) = 0.00038 mg a.s./L E_bC_{50} (72 h) = 0.00018 mg a.s./L (mean measured)	Reliability: 1	Wisk (1998) ECO 96-307
OECD Guideline 201, EC Guideline C3, and U.S. EPA Guideline 123-2	*Anabaena flos-aquae*	Picolinafen (^{14}C-labeled) (purity: 97.8 %)	E_rC_{50} (120 h) > 0.00039 mg a.s./L E_bC_{50} (120 h) = 0.00034 mg a.s./L (mean measured)	Reliability: 3	Barker et al. (1998)
OECD 201 and EC Guideline C3 (recovery test)	*Pseudokirchneriella subcapitata*	Picolinafen (purity: 97.8 %)	E_yC_{50} = 0.00017 mg/L (nominal)	Reliability: 1 Supplementary information	Barker (1999) ECO 99-001
OECD 201 and EC Guideline C3	*Pseudokirchneriella subcapitata*	Metabolite CL 153815* (purity: 100 %)	E_rC_{50} (72 h) > 50 mg/L E_bC_{50} (72 h) = 27 mg/L (mean measured)	Reliability: 1 Supplementary information	Drottar et al. (1998) ECO 97-353
OECD 201 (2006); EC 761/2009 C.3 Algal inhibition test	*Pseudokirchneriella subcapitata*	Metabolite CL 7693* (purity: 99.7 %)	E_rC_{50} (72 h) = 14 mg/L E_bC_{50} (72 h) = 1.84 mg/L (mean measured)	Reliability: 2 Supplementary information	Kley & Deierling (2011) 61321210
American Society for Testing and Materials (1990). Standard Guide for Conducting Static Toxicity Tests with Lemna gibba G3.	*Lemna gibba*	Picolinafen (^{14}C-labeled) (purity: 97.8 %)	E_rC_{50} (72 h) = 0.057 mg a.s./L E_bC_{50} (72 h) = 0.08 mg a.s./L (initial mean measured)	Reliability: 2	Barker (1998) ECO 97-161

*For further information on the structure of metabolites CL 153815 (picolinic acid) and CL 7693 (p-fluoroaniline), please refer to section 9.1

11.5.1 Acute (short-term) toxicity to fish

11.5.1.1 Study 1

Author:	Anonymous
Title:	Acute toxicity of AC 900.001 to Rainbow trout (*Oncorhynchus mykiss*) under Flow-through test conditions
Date:	1998
Doc ID:	ECO 96-309; abc 43439, WAT1999-514
Guidelines:	EPA Guideline 72-1(c), OECD Guideline 203, and EC Guideline C1
GLP:	Yes
Validity:	Valid
Previous evaluation:	In initial DAR (2000)

Materials and methods

Groups of twenty rainbow trout were exposed to technical grade AC 900001 (Lot Number CA 14113, 97.8 % pure) for 96 hours under flow-through test conditions. Test solutions were prepared and delivered to the test vessels by a proportional diluter system. A vehicle (acetone) blank was also tested in addition to a no-treatment control group. Nominal test concentrations for the 96-hour definitive test were, 0.0 (control), 0.0 (vehicle blank), 0.063, 0.13, 0.25, 0.50, and 1.0 mg as/L. These concentrations were chosen based on the lack of toxicity observed during a toxicity range-finding test and the limited water solubility of AC 900001 (i.e., 0.04 mg/L). The numbers of dead rainbow trout in each treatment were recorded at least once daily. The actual exposure concentrations were verified using a validated HPLC method.

Results and Discussion

The mean measured exposure concentrations of AC 900001 during the 96-hour test period were: 0.0 (control), 0.0 (vehicle blank), 0.051, 0.088, 0.15, 0.31, and 0.68 mg as/L (ppm). After 96 hours of exposure, there were no mortalities in the controls or any of the AC 900001 treatments. Based on the mean measured concentrations of AC 900001 during the 96 hour definitive test, the 96-hour LC50 and NOEC values were determined to be > 0.68 mg as/L and 0.68 mg as/L, respectively.

Conclusion

The 96-hour LC_{50} and NOEC values for Picolinafen in the rainbow trout were > 0.68 mg as/L and 0.68 mg as/L, respectively. The study is valid and reliable. It is considered relevant for classification purposes.

11.5.1.2 Study 2

Author:	Anonymous
Title:	Acute toxicity of AC 900.001 to Bluegill Sunfish (*Lepomis macrochirus*) under Flow-through test conditions
Date:	1998
Doc ID:	ECO 96-308; abc 43440, WAT 1999-515
Guidelines:	US EPA Guideline 72-1(a), OECD Guideline 203, and EC Guideline C1
GLP:	Yes
Validity:	Valid
Previous evaluation:	In initial DAR (2000)

Materials and methods

Groups of twenty bluegill sunfish were exposed to technical grade AC 900001 (Lot Number CA 14113, 97.8 % pure) for 96 hours under flow-through test conditions. Test solutions were prepared and delivered to the test vessels by a proportional diluter system. A vehicle (acetone) blank was also tested in addition to a no-treatment control group. Nominal test concentrations for the 96-hour definitive test were, 0.0 (control), 0.0 (vehicle blank), 0.063, 0.13, 0.25, 0.50, and 1.0 mg as/L. These concentrations were chosen based on the lack of toxicity observed during a toxicity range-finding test and the limited water solubility of AC 900001 (i.e., 0.04 mg/L). The numbers of dead bluegill sunfish in each treatment were recorded at least once daily. The actual exposure concentrations were verified using a validated HPLC method.

Results and Discussion

The mean measured exposure concentrations of AC 900001 during the 96-hour test period were: 0.0 (control), 0.0 (vehicle blank), 0.046, 0.084, 0.15, 0.24, and 0.57 mg as/L (ppm). After 96 hours of exposure, there were no mortalities in the controls or any of the AC 900001 treatments. Based on the mean measured concentrations of AC 900001 during the 96 hour definitive test, the 96-hour LC_{50} and NOEC values were determined to be > 0.57 mg as/L and 0.57 mg as/L, respectively.

Conclusions

The 96-hour LC_{50} and NOEC values for Picolinafen in the bluegill sunfish were > 0.57 mg as/L and 0.57 mg as/L, respectively. The 96-hour LC_{50} and NOEC values for AC 900001 in the bluegill sunfish were > 0.57 mg as/L and 0.57 mg as/L, respectively. The study is valid and reliable. It is considered relevant for classification purposes.

11.5.1.3 Study 3

Author: Anonymous

Title: Acute toxicity of CL 153815 to Rainbow trout, *Oncorhynchus mykiss*, under static test conditions

Date: 1998

Doc ID: ECO 97-351; 954-97-351 , WAT1999-518

Guidelines: US EPA Guideline 72-1(c), OECD Guideline 203, and EC Guideline C1

GLP: Yes

Validity: Valid

Previously evaluated: In initial DAR (2000)

Materials and methods

This study was conducted to evaluate the toxicity of CL 153815, the primary degradate of AC 900001, in a water/sediment system (See Annex IIA, Section 5, Point 7.2.1.3.2), to fish. Groups of twenty rainbow trout were exposed to CL 153815 (Lot Number CA 16281, 100 % pure) for 96 hours under static test conditions. Test solutions were prepared by mixing the test substance in fresh well water. Nominal test concentrations for the 96-hour definitive test were, 0.0 (control), 13, 22, 36, 60, and 100 mg/L. The numbers of dead rainbow trout in each treatment were recorded at least once daily. The actual exposure concentrations were verified using a validated HPLC method.

Results and Discussion

The mean measured exposure concentrations of CL 153815 during the 96-hour test period were: 0.0 (control), 13, 21, 35, 58, and 100 mg/L (ppm). After 96 hours of exposure, there were no mortalities in the control or any of the CL 153815 treatments. Based on the mean measured concentrations of CL 153815 during the 96 hour definitive test, the 96-hour LC_{50} and NOEC values were determined to be > 100 mg/L and 100 mg/L, respectively.

Conclusions

The 96-hour LC_{50} and NOEC values for CL 153815 in the rainbow trout were > 100 mg/L and 100 mg/L, respectively. The study is valid and reliable. As it is conducted with a metabolite of picolinafen, which shows lower toxicity than the parent, it is considered as supplementary information for classification purposes.

11.5.1.4 Study 4

Author: Anonymous

Title: Acute toxicity of CL 7693 to rainbow trout (*Oncorhynchus mykiss*) in a 96-hour static test

Date: 2011

Doc ID: 61323230

Guidelines: OECD 203 (1992); EC 440/2008 C.1 Acute Toxicity for Fish

GLP: Yes

Validity: Yes

Previous evaluation: Submitted for the purpose of renewal

Materials and methods

Test Material:	CL 7693
IUPAC Name:	4-fluoroaniline
Description:	Orange liquid (purity 99.7 %)
Lot/Batch #:	AC12214-129
Stability of test compound:	Considered to be sufficiently stable for purpose of study
Test organisms	
Species:	Rainbow trout (*Oncorhynchus mykiss*); mean length: 5.08 ± 0.38 cm; mean weight 1.26 ± 0.34 g
Length / Weight:	mean length: 5.08 ± 0.38 cm; mean weight 1.26 ± 0.34 g
Food:	None during study
Treatments	
Test concentrations:	4.3, 9.4, 21, 45 and 100 mg CL7693/L
Control:	Reconstituted water
Test design	
Replication:	1
No. of organisms/treatment:	7
Exposure regime:	static
Environmental conditions	
Temperature:	13 – 15 °C
Oxygen concentration:	92 – 100 % air saturation value
Photoperiod:	16:8 L:D (30 min/dawn/dusk period; 480 – 1060 lux)
pH:	7.6 – 8.0
Observations	
Mortality/ sublethal effects:	2, 24, 48, 72 and 96 hours following introduction of fish
Environmental conditions:	daily measurements (temperature, oxygen, pH)
Analysis of test item:	0 and 24 (at nominal 45 and 100 mg/L) resp. 96 hours (at nominal 9.4 and 21 mg/L); HPLC-method (liquid chromatography)
Statistics:	The 96-hour LC_{50} was calculated by Probit analysis (using linear weighted regression). The NOEC, LOEC, LC_0 and LC_{100} were determined directly from the raw data. Statistical analysis was performed with ToxRat Professional (Version 2.10.05, ToxRat® Solutions GmbH).
In-life dates:	22 – 26 November 2010

Results and Discussion

The biological results of the study are summarised in the following table.

Table B.11.5-1: Mortality and sublethal effects of rainbow trout (*O. mykiss*) exposed to CL 7693 in a 96 h static acute toxicity test

Nominal concentration (mg/L)	**Mean measured concentration (mg/L)**	**Mortality / Sublethal effects***					
		Exposure time (hours)					
		0	**2**	**24**	**48**	**72**	**96**
Control	-	0	0	0	0	0	0
4.3	not measured	0	0	0	0	0	0
9.4	7.4	0	0	0	0	0	0
21	16	0	0	1	2 (5[#])	2	2
45	37	0	2 (5)	7	7	7	7
100	87	0 (7)	4 (3)	7	7	7	7

* no. of fish showing symptoms are given in brackets

[#] strong ventilation of fish observed after 48 h of test duration, not observed at 72 and 96 hours.

Analysis of the test concentrations revealed test item recoveries of 73 to 87 % of the nominal values at the start of the test (just before introduction of the fish) at nominal 9.4, 21, 45, and 100 mg/L. After 96 hours test duration 76 to 87 % of the nominal values were found. Thus, the measured concentrations were partly below 80 % of the nominal values and the results were related to mean measured concentrations of the test item.

Based on the test results the 96-hour LC_{50} was determined to be 19.9 mg/L (mean measured), its 95 % confidence interval could not be determined. The 96-hour NOEC was determined to be 16 mg/L (mean measured).

Conclusions

The 96-hour LC_{50} of CL 7693 for Rainbow Trout (*Oncorhynchus mykiss*) was determined to be 19.9 mg/L. The 96-hour NOEC and LOEC values were determined to be 7.4 and 16 mg test item/L, both values based on mean measured test concentrations. The study is valid and reliable. As it is conducted with a metabolite of picolinafen, which shows lower toxicity than the parent, it is considered as supplementary information for classification purposes.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

11.5.2.1 Study 1

Author: Wisk, J.D.; Sword, M.C.; Steward, S. and Gardner, C.

Title: Acute toxicity of AC 900001 to *Daphnia magna* under static test conditions

Date: 1998

Doc ID: ECO 96-182 , WAT1999-521

Guidelines: US EPA Guideline 72-2, OECD 202 Part A, and EC Guideline C2

GLP: Yes

Validity: Valid

Materials and methods

Groups of twenty *Daphnia magna*, less than 24 hours of age, were exposed to technical grade picolinafen (AC 900001, Lot Number CP 29327, 98.7 % pure) for 48 hours under static test conditions. Test solutions were prepared by first, preparing stock solutions of the test substance in acetone, and then adding 0.1 mL of the appropriate stocks to 1 L of dilution water. A vehicle blank was also prepared and tested at 0.1 mL of acetone/L. Nominal test concentrations for the 48-hour definitive test were, 0.0 (control, 0.0 (vehicle blank), 0.063, 0.13, 0.25, 0.50, and 1.0 mg as/L. These concentrations were chosen based on the lack of toxicity observed during a toxicity range-finding test and the limited water solubility of AC 900001 (i.e., 0.04 mg/L). The number of immobile daphnids in each treatment was recorded at least once daily. The actual exposure concentrations were verified using a validated HPLC method.

Results and Discussion

The mean measured exposure concentrations of AC 900001 during the 48-hour test period were: 0.0 (control), 0.0 (vehicle blank), 0.051, 0.071, 0.14, 0.22, and 0.45 mg as/L (ppm). After 48 hours of exposure, there were no dead or immobile daphnids in the controls or any of the AC 900001 treatments. Based on the mean measured concentrations of AC 900001 during the 48 hour definitive test, the 48-hour EC_{50} and NOEC values were determined to be > 0.45 mg as/L and 0.45 mg as/L, respectively.

Conclusions

The 48-hour EC50 and NOEC values for picolinafen in Daphnia magna were > 0.45 mg as/L and 0.45 mg as/L, respectively. The study is valid and reliable. It is relevant for classification purposes.

11.5.2.2 Study 2

Author:	Drottar, K.R.; Krueger, H.O.; MacGregor, J.A. and Olivieri, C.E.
Title:	Acute toxicity of CL 153815 to *Daphnia magna* under static test conditions
Date:	1998
Doc ID:	ECO 97-352 , WAT1999-520
Guidelines:	US EPA Guideline 72-2, OECD 202 Part A, and EC Guideline C2
GLP:	Yes
Validity:	Valid
Previous evaluation:	In initial DAR (2000)

Materials and methods

This study was conducted to evaluate the toxicity of CL 153815, the primary degradate of AC 900001 in a water/sediment system (See Annex IIA, Section 5, Point 7.2.1.3.2), to aquatic invertebrates. Groups of twenty *Daphnia magna*, less than 24 hours of age, were exposed to CL 153815 (Lot Number CA 16281, 100 % pure) for 48 hours under static test conditions. Test solutions were prepared by first, preparing stock solutions of the test substance in fresh well water, and then adding aliquots of the appropriate stocks to dilution water. Nominal test concentrations for the 48-hour definitive test were, 0.0 (control), 6.3, 13, 25, 50, and 100 mg/L. The number of immobile daphnids in each treatment was recorded at least once daily. The actual exposure concentrations were verified using a validated HPLC method.

Results and Discussion

The mean measured exposure concentrations of CL 153815 during the 48-hour test period were: 0.0 (control), 6.0, 12, 25, 49, and 98 mg/L (ppm). After 48 hours of exposure, there were no dead or immobile daphnids in the control and the 6.0 mg/L treatment. After 48-hours of exposure, *Daphnia* mortality in the 12,

25, 49, and 98 mg/L treatments were 10, 10, 25, and 40 %, respectively. Based on the mean measured concentrations of CL 153815 during the 48 hour definitive test, the 48-hour EC_{50} and NOEC values were determined to be > 98 mg/L and 6.0 mg/L, respectively.

Conclusions

The 48-hour EC_{50} and NOEC values for CL 153815 in *Daphnia magna* were > 98 mg/L and 6.0 mg/L (mean measured), respectively. The study is valid and reliable. As it is conducted with a metabolite of picolinafen, which shows lower toxicity than the parent, it is considered as supplementary information for classification purposes.

11.5.2.3 Study 3

Author:	Kley A., Deierling T.
Title:	Acute toxicity of CL7693 to *Daphnia magna* in a semi static 48-hour immobilisation test
Date:	2011
Doc ID:	61322220
Guidelines:	OECD 202 (2004); EC 440/2008 C.2 Daphnia sp. Acute Immobilisation Test
GLP:	Yes
Validity:	Valid
Previous evaluation:	Submitted for the purpose of renewal

Materials and methods

Test Material:	CL 7693
IUPAC Name:	4-fluoroaniline
Description:	Orange liquid (purity 99.7 %)
Lot/Batch #:	AC12214-129
Stability of test compound:	Considered sufficiently stable for purpose of study
Test organisms	
Species:	*Daphnia magna* (Straus); age: 3.75 to 19.5 hours old
Strain:	Clone 5
Source:	In-house culture
Food:	None during study
Treatments	
Test concentrations:	0.019, 0.042, 0.093, 0.20 and 0.45 mg CL7693/L
Control:	Reconstituted water
Test design	
Replication:	4
No. of organisms/treatment:	20
Exposure regime:	semi-static
Environmental conditions	
Temperature:	20 °C

Oxygen concentration: 8.2 – 9.1 mg/L

Photoperiod: 16:8 L:D (650 – 830 lux)

pH: 7.9 – 8.0

Observations

Immobility: 24 and 48 hours following introduction of daphnids

Environmental conditions: measurement of all fresh and aged test media (temperature, oxygen, pH)

Analysis of test item: 0, 24 and 48 hours (fresh and aged test media); HPLC-method (liquid chromatography)

Statistics: The 48-hour EC_{50} was calculated by Probit analysis. The 48-hour NOEC and LOEC values were determined directly from the raw data. Statistical analysis was performed with ToxRat Professional (Version 2.10.05, ToxRat® Solutions GmbH).

In-life dates: 05 – 07 July 2011

Results and Discussion

The biological results of the study are summarised in the following table.

Table B.11.5-2: Immobility of *Daphnia magna* exposed to CL 7693 in a 48 h semi-static acute toxicity test

Nominal concentration (mg /L)	**% of immobilised *Daphnia* after 24 and 48 hours**	
	24 hours	**48 hours**
Control	0	0
0.019	0	0
0.042	0	0
0.093	0	5
0.20	0	10
0.45	10	100

Analytical analysis revealed recoveries of 54 to 121 % of the nominal test concentrations at the start of the test and at test medium renewal. In the aged test media, 70 - 120 % of the nominal values were found. Since test item recoveries of <80 % only occurred at the lowest test concentration of nominal 0.019 mg/L, which is below the 24- and 48-hour NOEC of the test and thus not relevant for the calculation/determination of the study endpoints, the study endpoints can be related to nominal test item concentrations. Thus, all reported results refer to nominal concentrations.

Based on the test results the 48-hour EC_{50} was determined to be 0.254 mg/L, its 95 % confidence interval could not be determined. The 48-hour NOEC was determined to be 0.20 mg/L.

Conclusions

The toxic effect of the test item CL 7693 to *Daphnia magna* was assessed in a semi-static dose-response test. The 48-hour EC_{50} was calculated to be 0.254 mg test item/L. The 48-hour NOEC and LOEC values were

determined to be 0.042 and 0.093 mg test item/L, respectively. The study is valid and reliable. It is considered relevant for classification purposes.

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

11.5.3.1 Study 1

Author: Wisk, J.; Barker, C.; Hicks, S. and Stewart, S.

Title: Effect of AC 900001 on Growth of the Green Alga, *Selenastrum capricornutum*

Date: 1998

Doc ID: ECO 96-307 , WAT1999-525

Guidelines: OECD 201 and EC Guideline C3

GLP: Yes

Validity: Valid

Previous evaluation: In initial DAR (2000)

Materials and methods

A 72-hour toxicity test was conducted with the green alga, *Selenastrum capricornutum* by exposing the organisms to ^{14}C-radioloabeled picolinafen (AC 900001, Lot Number AC 10011-110, 97.8 % radiopurity) under static test conditions. Test solutions were prepared by first, preparing stock solutions of the test substance in acetone, and then adding appropriate stocks to algal media. A vehicle blank was also prepared and tested, as was a no-treatment (algal media only) control. Nominal test concentrations for the 72-hour definitive test were, 0.0 (control), 0.0 (vehicle blank), 0.05, 0.10, 0.20, 0.40, and 0.80 µg as/L. The number of algal cells per mL of media in each treatment was determined once daily. The actual exposure concentrations of AC 900001 were verified by liquid scintillation counting.

Results and Discussion

The measured concentrations of ^{14}C-AC 900001 equivalents at time 0 were: 0.0 (control), 0.0 (vehicle blank), 0.0685, 0.0984, 0.163, 0.335, and 0.728 µg/L. The measured concentrations of ^{14}C-AC 900001 equivalents at 72 hours were: 0.0 (control), 0.0 (vehicle blank), 0.0679, 0.0968, 0.165, 0.348, and 0.724 µg/L. The maximum deviation between the time 0 and 72 hours was 3.8 %. The mean measured exposure concentrations of ^{14}C-AC 900001 equivalents during the 72-hour test period were: 0.0 (control), 0.0 (vehicle blank), 0.068, 0.098, 0.16, 0.34, and 0.73 µg as equivalents./L (ppb).

The effect of AC 900001 on algal cell growth after 72 hours of exposure is summarised in table below.

Table 50: Effect of picolinafen on algal cell density after 72 hours of exposure

Treatment	72-Hour Mean Cell Density (cells/mL)
Control	110 x 10^4
Vehicle Blank	120 x 10^4
0.068 µg/L	120 x 10^4
0.098 µg/L	110 x 10^4

0.16 µg/L	64 x 10^4
0.34 µg/L	12 x 10^4
0.73 µg/L	2.9 x 10^4

Based on the mean measured concentrations of picolinafen during the 72 hour definitive test, the 72-hour EC_{50} for biomass (E_bC_{50}; based on area under the growth curve) and NOEC for biomass were determined to be 0.18 µg as/L and 0.068 µg as/L, respectively. The EC_{50} based on growth rate (E_rC_{50}) and NOEC for growth rate were 0.38 µg as/L and 0.098 µg as/L.

Conclusions

The most sensitive endpoint in *S. capricornutum* to AC 900001 was effects on biomass. Based on this endpoint, the 72-hour EC_{50} and NOEC values were determined to be 0.18 µg ^{14}C-AC 900001 equivalents/L and 0.068 µg ^{14}C-AC 900001 equivalents/L, respectively. The 72-hour EC_{50} and NOEC based on growth rate were 0.38 µg ^{14}C-AC 900001 equivalents/L and 0.098 µg ^{14}C-AC 900001/L, respectively.

In the highest test concentration 41 % effect on growth rate could be seen. The mean coefficient of variation for section-by-section specific growth rates (control) is 16.5 % and the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures is 2.4 %. Therefore, this test is also valid according to validity criteria of current OECD Guideline 201 (2006). The study is valid and reliable. It is relevant for classification purposes.

11.5.3.2 Study 2

Author: Barker, C.L.; Hicks, S. and Hurshman; B.

Title: Effect of AC 9000001 on the Growth of *Anabaena flos-aquae*

Date: 1998

Doc ID: ECO 97-163 , WAT1999-522

Guidelines: OECD Guideline 201, EC Guideline C3, and U.S. EPA Guideline 123-2

GLP: Yes

Validity: Not valid

Previous evaluation: In initial DAR (2000)

Materials and methods

A 120-hour toxicity test was conducted with the blue-green alga, *Anabaena flos-aquae* by exposing the organisms to ^{14}C-radioloabeled AC 900001 (Lot Number AC 10011-110, 97.8 % radiopurity) under static test conditions. Test solutions were prepared by first, preparing stock solutions of the test substance in acetone, and then adding appropriate stocks to algal media. A vehicle blank was also prepared and tested, as was a no-treatment (algal media only) control. Nominal test concentrations for the 120-hour definitive test were, 0.0 (control), 0.0 (vehicle blank), 0.0085, 0.017, 0.033, 0.065, 0.13, 0.25, and 0.50 mg as/L. The number of algal cells per mL of media in each treatment were determined once daily. The actual exposure concentrations of AC 900001 were verified by liquid scintillation counting.

Results and Discussion

The mean measured exposure concentrations of AC 900001 during the 120-hour test period were: 0.0 (control), 0.0 (vehicle blank), 0.0084, 0.017, 0.033, 0.063, 0.12, 0.22, and 0.39 mg as equivalents/L (ppm).

The effect of AC 900001 on algal cell growth after 120 hours of exposure is summarised in Table 51.

Table 51: Effect of AC 900001 on Algal Cell Density After 120 Hours of Exposure

Treatment	**72-Hour Mean Cell Density (cells/mL)**
Control	93 x 10^4
Vehicle Blank	86 x 10^4
0.0084 mg/L	88 x 10^4
0.017 mg/L	75 x 10^4
0.033 mg/L	64 x 10^4
0.063 mg/L	66 x 10^4
0.12 mg/L	60 x 10^4
0.22 mg/L	52 x 10^4
0.39 mg/L	40 x 10^4

Based on the mean measured concentrations of ^{14}C-AC 900001 equivalents during the 120-hour definitive test, the 120-hour EC_{50} for biomass (E_bC_{50}; based on area under the growth curve) and NOEC for biomass were determined to be 0.34 mg ^{14}C-AC 900001 equivalents/L and 0.063 mg ^{14}C-AC 900001 equivalents/L, respectively. The EC_{50} based on growth rate (E_rC_{50}) and NOEC for growth rate were > 0.39 mg ^{14}C-AC 900001 equivalents/L and 0.063 mg ^{14}C-AC 900001 equivalents/L.

Conclusions

The most sensitive endpoint in *A. flos-aquae* to AC 900001 was effects on biomass. Based on this endpoint, the 120-hour EC_{50} and NOEC values were determined to be 0.34 mg ^{14}C-AC 900001 equivalents /L and 0.063 mg ^{14}C-AC 900001 equivalents/L, respectively. The 120-hour EC_{50} and NOEC based on growth rate were > 0.39 mg ^{14}C-AC 900001 equivalents /L and 0.063 mg ^{14}C-AC 900001 equivalents/L, respectively.

According to current OECD Guideline 201 (2006) this study is no longer valid due to the following shortcomings: 1. the mean coefficient of variation for section-by-section specific growth rates in the control cultures is 21.2 % and therefore exceeds the validity criterion of 35 %. 2. The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures is 88.1 % and therefore exceeds the validity criterion of 10 %. 3. The initial cell numbers were only detected in control replicates but not for treated vessels. Initial biomass is low and variability between replicates of the control range from 1100 to 7800 cells per mL. Whereas, the recommended initial biomass for *Anabaena flos-aquae* is 10^4 cells/mL. The study is considered as not reliable. It is not relevant for classification purposes.

11.5.3.3 Study 3

Author:	Barker, C.L. and Kranzfelder, J.A.
Title:	Recovery Potential of the Green Alga, *Selenastrum capricornutum*, following 72 hours of Exposure to AC 900001
Date:	1999
Doc ID:	ECO 99-001 , WAT1999-523
Guidelines:	OECD 201 and EC Guideline C3
GLP:	No
Validity:	Valid
Previous evaluation:	In initial DAR (2000)

Materials and methods

This study was conducted to evaluate the potential for recovery of algal populations after exposure toxic concentrations of picolinafen. A 72-hour static exposure to picolinafen (AC 900001 technical, Batch Number 001, 97.8 % pure) was followed by a 14-day recovery period. The recovery period was initiated with the extraction of algal cells from treatment solutions and placing them into untreated algal media.

Nominal test concentrations for the 72-hour exposure were 0.0 (vehicle blank; 0.1 mL/L dimethylformamide), 0.13, 0.25, 0.50, 1.0, and 2.0 µg AC 900001/L. Cell counts were made daily during the 72-hour exposure. After 72 hours of exposure, aliquots of the exposed cells were used to inoculate untreated algal media at a targeted concentration of 3000 cells/mL.

Results and Discussion

After 72-hours of exposure to AC 900001 technical, percent inhibition ranged from 25 % at 0.13 µg/L to >95 % at concentrations $\geq$ 0.50 µg/L. the 72-hour EC_{50} value based on biomass (i.e., area under the growth curves) was 0.17 µg/L (i.e., similar to the results from the guideline study with AC 900001 (see study 1).

During the recovery period, cell growth followed a similar pattern for the vehicle blank and the 0.13 and 0.25 µg/L treatments, with a 2-day lag period followed by exponential growth. At 0.50 µg/L, algal cells remained in the lag phase of growth until day 6, when exponential growth began. Cells exposed to 0.50 µg/L reached control cell densities by day 9 of the recovery period. At 1.0 and 2.0 µg/L, the lag phase lasted until approximately day 7 of the recovery period. Cell densities in these two treatments reached control densities by day 10 of the recovery period.

Growth rate was calculated between adjacent time points during the first ten days of the recovery period. During the first two days of the recovery period, cell growth was slower in all treatments in comparison to the controls. However, beginning with the day 2 to 3 time period, cell growth rate was equivalent in all treatments in comparison to the controls.

Conclusions

The results from this modified laboratory study indicate that upon removal of picolinafen from the test systems algal populations exposed to concentrations as high as 2.0µg/L will fully recover. These results indicate that AC 900001 is primarily algalstatic (i.e., inhibits growth) rather than algalcidal (i.e., kills algae) in its mode of action.

The study is valid and reliable. It is considered as supplementary information for classification purposes.

11.5.3.4 Study 4

Author: Drottar, K.R.; Sutherland, C.A.; Krüeger, H.O. and Olivieri, C.E.

Title: Effect of CL 153815 on growth of the green alga, *Selenastrum capricornutum*

Date: 1998

Doc ID: ECO 97-353 , WAT1999-524

Guidelines: OECD 201 and EC Guideline C3

GLP: Yes

Validity: Valid

Previous evaluation: In initial DAR (2000)

Materials and methods

This study was conducted to evaluate the toxicity of CL 153815, the primary degradate of AC 900001 in a water/sediment system (See Annex IIA, Section 5, Point 7.2.1.3.2), to green algae. A 72-hour toxicity test was conducted with the green alga, *Selenastrum capricornutum* by exposing the organisms to CL 153815 (Lot Number CA 16281, 100 % pure) under static test conditions. Test solutions were prepared by first, preparing the highest test concentration of the test substance in freshwater algal medium, and then preparing the remaining test solutions by proportional dilution of the high concentration test solution with algal media. A no-treatment (algal media only) control was also tested. Nominal test concentrations for the 72-hour definitive test were, 0.0 (control), 1.6, 3.1, 6.3, 13, 25, and 50 mg/L. The number of algal cells per mL of media in each treatment was determined once daily. The actual exposure concentrations of CL 153815 were verified using a validated HPLC method (Cyanamid Study Number 954-98-412).

Results and Discussion

The mean measured exposure concentrations of CL 153815 during the 72-hour test period were: 0.0 (control), 1.5, 3.1, 6.1, 12, 25, and 50 mg/L (ppm).

The effect of CL 153815 on algal cell growth after 72 hours of exposure is summarised in

Table 52.

Table 52: Effect of CL 153815 on Algal Cell Density After 72 Hours of Exposure

Treatment	72-Hour Mean Cell Density (cells/mL)
Control	1,412,721
1.5 mg/L	1,237,527
3.1 mg/L	1,302,476
6.1 mg/L	1,125,709
12 mg/L	1,034,054
25 mg/L	692,472
50 mg/L	196,406

Based on the mean measured concentrations of CL 153815 during the 72-hour definitive test, the 72-hour EC_{50} for biomass (E_bC_{50}; based on area under the growth curve) and NOEC for biomass were determined to

be 27 mg/L and 12 mg/L, respectively. The EC_{50} based on growth rate (E_rC_{50}) and NOEC for growth rate were > 50 mg/L and 12 mg/L, respectively.

Conclusions

The most sensitive endpoint in *S. capricornutum* to CL 153815 was effects on biomass. Based on this endpoint, the 72-hour EC_{50} and NOEC values were determined to be 27 mg/L and 12 mg/L, respectively. The 72-hour EC_{50} and NOEC based on growth rate were > 50 mg/L and 12 mg/L, respectively.

The mean coefficient of variation for section-by-section specific growth rates (control) is 14.5 % and the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures is 3.9 %. Therefore, this test is also valid according to validity criteria of current OECD Guideline 201 (2006). The study is valid and reliable. As it is conducted with a metabolite which shows lower toxicity than the parent picolinafen, it is considered as supplementary information for classification purposes.

11.5.3.5 Study 5

Author:	Kley A., Deierling T.
Title:	Toxicity of CL 7693 to *Pseudokirchneriella subcapitata* in an algal growth inhibition test
Date:	2011
Doc ID:	61321210
Guidelines:	OECD 201 (2006); EC 761/2009 C.3 Algal inhibition test
GLP:	Yes
Validity:	Acceptable
Previous evaluation:	Submitted for the purpose of renewal

Materials and methods

Test Material: CL 7693

IUPAC Name: 4-fluoroaniline

Description: Orange liquid (purity 99.7 %)

Lot/Batch #: AC12214-129

Stability of test compound: Considered sufficiently stable for purpose of study

Test organisms

Species: *Pseudokirchneriella subcapitata*

Strain: Strain No.: 61.81 SAG

Source: Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen, 37073 Göttingen, Germany

Treatments

Test concentrations: 0.10, 0.32, 1.0, 3.2 and 20 mg CL7693/L

Control: Reconstituted water

Test design

Replication:	3 replicates for test item treatments, 6 control replicates
Inoculated algal cells:	5000
Exposure regime:	static

Environmental conditions

Temperature:	22 – 23 °C
Photoperiod:	Continuous illumination (range: 5610 – 5930 lux)
pH:	8.0 – 9.4

Observations

Algal cell density:	24, 48, and 72 hours after inoculation of algae
Environmental conditions:	daily measurements of temperature, pH measurement at test start and end
Analysis of test item:	0 and 72 hours; HPLC-method (liquid chromatography)

Statistics:	The 72-hour E_yC_{50} values were calculated by Probit analysis. The 72-hour NOEC and LOEC values were determined by Williams t-test. Statistical analysis was performed with ToxRat Professional (Version 2.10.05, ToxRat® Solutions GmbH).
In-life dates:	08 – 11 November 2010

Results and Discussion

At the start of the test, 74 to 89 % of the nominal test item concentrations were analytically determined in the test media of nominal 1.0, 3.2, and 20 mg/L (test concentrations above and including the NOEC). Test media of lower test concentrations were not analysed. After 72 hours test duration, 28 to 44 % of the nominal values were determined. Since the test item concentrations were not stable during the test duration all reported results refer to geometric mean measured test concentrations. The nominal concentrations of 1.0, 3.2 and 20 mg/L corresponded to geometric mean measured concentrations of 0.487, 1.57 and 11.98 mg/L, respectively.

The most sensitive parameter of the test was the yield of the algae. At the test concentrations of nominal 1.0, 3.2 and 20 mg/L, inhibitions of yield of 2.5, 46 and 92 % were observed after 72 hours of test duration, respectively. The microscopic examination of the shape of the algal cells after 72 hours did not show any difference between the algae that had been growing at the nominal test concentration of 20 mg test item/L and the algal cells in the control.

The resulting EC_x, NOEC, and LOEC values (based on mean measured concentrations of the test item) are summarised in the following table.

Table 53: Effects on growth rate and yield of *Pseudokirchneriella subcapitata* following exposure to CL 7693

Parameter (0 – 72 hours)	**Growth rate**	**Yield**
72-hour EC_{50} (mg/L):	14.0*	1.84
95 % confidence limits	11.6 – 17.7	1.55 – 2.31
72-hour EC_{10} (mg/L):	1.48	0.504
95 % confidence limits	0.937 – 2.04	0.313 – 0.664
72-hour NOEC (mg/L):	0.487	0.487
72-hour LOEC (mg/L):	1.57	1.57

* extrapolated value

Conclusions

The influence of CL 7693 on the growth of the freshwater green algae *Pseudokirchneriella subcapitata* was assessed in a static dose-response test. The 72-hour E_rC_{50} value was calculated to be 14.0 mg test item/L (extrapolated) and the 72-hour E_yC_{50} was calculated to be 1.84 mg test item/L. The 72-hour NOE_rC and the 72-hour NOE_yC were determined to be 0.487 mg test item/L and the associated 72-hour LOE_rC and LOE_yC is 1.57 mg test item/L.

The study is acceptable in spite of the following shortcoming: Due to the enlarged spacing factor of nominal 6.25 between the test item concentrations of 3.2 and 20 mg/L, provoking 46 and 92 % inhibition, respectively, the slope of the concentration-effect-relationship may not be correctly described by the study. An inhibition of 92 % may be seen as a complete growth inhibition, which could have been already provoked by a lower test item concentration, e.g. 10 mg/L. Nevertheless a $NOEC_{(72\,h,\ stat.,\ mean\ meas.)}$ = 0.487 mg test item/L can be derived from the study. However, considering no effects up to 0.487 mg/L and that at 3.2 mg/L growth inhibition was 46 %, and therefore close to 50 %, it can be derived from this study, that CL 7693 (metabolite of picolinafen), is less toxic than the parent picolinafen. The study is considered reliable with restrictions. As it is conducted with a metabolite which shows lower toxicity than the parent picolinafen, it is considered as supplementary information for classification purposes.

11.5.3.6 Study 6

Author: Barker, C.; Hicks, S.L. and Hurshman, B.A.

Title: Effect of AC 9000001 on the Growth of *Lemna gibba* G3

Date: 1998

Doc ID: ECO 97-161, WAT1999-527

Guidelines: American Society for Testing and Materials (1990). Standard Guide for Conducting Static Toxicity Tests with Lemna gibba G3.

GLP: Yes

Validity: Valid

Previous evaluation: In initial DAR (2000)

Materials and methods

A 14-day toxicity test was conducted with the duckweed, *Lemna gibba* by exposing the organisms to ^{14}C-radioloabeled picolinafen (AC 900001, Lot Number AC 10011-110, 97.8 % radiopurity) under static test conditions. Test solutions were prepared by first, preparing stock solutions of the test substance in acetone, and then adding appropriate stocks to growth media. A vehicle blank was also prepared and tested, as was a no-treatment (growth media only) control. Nominal test concentrations for the 14-day definitive test were, 0.0 (control), 0.0 (vehicle blank), 8.5, 17, 33, 65, 130, and 250 µg as/L. The actual exposure concentrations of AC 900001 were verified by liquid scintillation counting.

The test was initiated with the addition of the test organisms to the test vessels containing test solution. A total of 14 fronds were added to each of the no-treatment and vehicle blank test vessels, while 15 fronds were added to each of the treatment test vessels. Each treatment and control contained four replicates. The number of fronds in each test vessel was determined on test days 0, 2, 4, 6, 9, 11, and 14. Observations of necrosis, chlorosis, frond death and changes in colour were made at each observation day. On test day 14, the duckweed was removed from each test vessel and biomass (i.e. dry weight) was determined.

Results and Discussion

The effect of the various treatments on frond number and biomass (dry weights) after 14 days of exposure are summarised in table below.

Table 54: Effect of picolinafen on frond number of dry weight of Lemna gibba after 14 days of exposure

Treatment[a]	Mean Frond Number	Mean Dry Weight (g)
Control	630	0.1086
vehicle blank	625	0.1079
7.2 µg/L	626	0.1104
14 µg/L	568*	0.1123
27 µg/L	507*	0.1021
59 µg/L	308*	0.0657*
120 µg/L	103*	0.0297*
210 µg/L	79*	0.0228*

[a]Concentrations represent day 0 concentrations of ^{14}C-AC 900001 equivalents/L.
*Statistically different from the controls.

Based on frond counts, the 14-day EC_{25} and EC_{50} values were 31 and 57 µg ^{14}C-AC 900001 equivalents/L, respectively. There was a statistically significant reduction in frond counts in all concentrations ≥ 14 µg/L. Therefore, the NOEC based on frond counts was 7.2 µg ^{14}C-AC 900001 equivalents/L.

Based on biomass, the 14-day EC_{25} and EC_{50} values were 46 and 80 µg ^{14}C-AC 900001 equivalents/L, respectively. There was a statistically significant reduction in biomass in all concentrations ≥ 59 µg/L. Therefore, the NOEC based on frond counts was 27 µg ^{14}C-AC 900001 equivalents/L.

Conclusions

The most sensitive endpoint in *Lemna gibba* to picolinafen was effects on frond number. Based on this endpoint, the 14-day EC_{50} and NOEC values were determined to be 57 µg ^{14}C-AC 900001 equivalents/L and 7.2 µg ^{14}C-AC 900001 equivalents/L, respectively. It should be noted that the study was accepted as valid in the initial EU peer review and thus the EC_{50} based on frond number of 0.057 mg as/L (meas. ini. 14 d) included in the endpoint list in the European Commission review report for picolinafen (Picolinafen SANCO/1418/2001-final, 18 September 2002). According to current OECD Guideline 221 (2006) "a semi-static test regime is recommended, if a preliminary stability test shows that the test substance concentration cannot be maintained (i.e. the measured concentration falls below 80 % of the measured initial concentration) over the test duration (7 days)." In the submited study with *Lemna* mean recovery of test substance decreased to 54 % after 14 days, which would trigger a semi-static test. The study is still considered valid and reliable with restrictions. It is relevant for classification purposes.

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

No data available.

11.6 Long-term aquatic hazard

Table 55: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
OECD Guideline 204	*Oncorhynchus mykiss*	Picolinafen (purity: 97.8 %)	NOEC (28 d) = 0.094 mg a.s./L (mean measured)	Reliability: 1 Only considered as supplementary information, because OECD 204 is not considered as adequate test for long-term aquatic hazard	Anonymous 27 (1999) ECO 97-162
U.S. EPA 72-4(a) and OECD 210	*Oncorhynchus mykiss*	Picolinafen (purity: 97.8 %)	NOEC (95 d) = 0.0064 mg a.s./L (mean measured)	Key study Reliability: 1	Anonymous 28 (1999) ECO 97-310
U.S. EPA 72-4(b) and OECD 202, Part B	*Daphnia magna*	Picolinafen (purity: 97.8 %)	NOEC (21 d) = 0.00706 mg a.s./L (mean measured)	Key study Reliability: 1	Barker (1998) ECO 97-164
OECD 201 and EC Guideline C3	*Pseudokirchneriella subcapitata*	Picolinafen (^{14}C-labeled) (purity: 97.8 %)	NOE_rC (72 h) = 0.000098 mg a.s./L (mean measured)	Key study Reliability: 1	Wisk (1998) ECO 96-307
OECD	*Anabaena*	Picolinafen	NOE_rC (120 h) =	Reliability: 3	Barker et al. (1998)

Guideline 201, EC Guideline C3, and U.S. EPA Guideline 123-2	*flos-aquae*	(^{14}C-labeled) (purity: 97.8 %)	0.000063 mg a.s./L (mean measured)		
OECD 201 and EC Guideline C3	*Pseudokirch neriella subcapitata*	Metabolite CL 153815* (purity: 97.8 %)	NOE_rC (72 h) = 12 mg/L (mean measured)	Reliability 1 Supplementary information	Drottar et al. (1998) ECO 97-353
OECD 201 (2006); EC 761/2009 C.3 Algal inhibition test	*Pseudokirch neriella subcapitata*	Metabolite CL 7693* (purity: 97.8 %)	NOE_rC (72 h) = 0.487 mg/L (mean measured)	Reliability: 2 Supplementary information	Kley & Deierling (2011) 61321210
American Society for Testing and Materials (1990). Standard Guide for Conducting Static Toxicity Tests with Lemna gibba G3.	*Lemna gibba*	Picolinafen (^{14}C-labeled) (purity: 97.8 %)	NOErC (72 h) = 0.0072 mg a.s./L (initial mean measured)	Reliability: 2	Barker (1998) ECO 97-161
BBA Draft Guideline "Effects of plant protection products on the sediment-dwelling larvae of *Chironomus repress* in a water-sediment system, and ASTM Guidelines	*Chironomus riparius*	Picolinafen (^{14}C-labeled) (purity: 97.8 %)	NOEC (10 d) = 0.18 mg a.s./L (initial mean measured)	Reliability: 1	Wisk (1998) ECO 96-310

*For further information on the structure of metabolites CL 153815 (picolinic acid) and CL 7693 (p-fluoroaniline), please refer to section 9.1

11.6.1 Chronic toxicity to fish

11.6.1.1 Study 1

Author: Anonymous

Title: Toxicity of AC 900001 to Rainbow trout (*Oncorhynchus mykiss*) in a Flow-through Prolonged Toxicity Test

Date: 1999

Doc ID: ECO 97-162; abc 43976 , WAT1999-516

Guidelines: OECD Guideline 204

GLP: Yes

Validity: Valid

Previous evaluation: In initial DAR (2000)

Materials and methods

Groups of twenty rainbow trout were exposed to technical grade AC 900001 (Lot Number CA 14113, 97.8 % pure) for 28 days under flow-through test conditions. Test solutions were prepared and delivered to the test vessels by a proportional diluter system. A vehicle (acetone) blank was also tested in addition to a no-treatment control group. Nominal test concentrations for the 28-day definitive test were, 0.0 (control), 0.0 (vehicle blank), 0.0063, 0.013, 0.025, 0.050, and 0.10 mg as/L. The numbers of dead rainbow trout in each treatment were recorded throughout the definitive test. After 28 days of exposure, effects of the test substance on growth (i.e., wet weights, standard lengths and total lengths) were evaluated. The actual exposure concentrations were verified during the test using a validated HPLC method.

Results and Discussion

The mean measured exposure concentrations of AC 900001 during the 28-day test period were: 0.0 (control), 0.0 (vehicle blank), 0.0064, 0.012, 0.021, 0.054, and 0.094 mg as/L (ppm). The mean measured concentrations ranged from 84 to 108 % of the targeted nominal concentrations.

After 28 days of exposure there were no mortalities in any treatment or control group. In addition, no sublethal adverse behavioural effects were observed. At test termination, there were no statistical differences in the mean standard lengths, mean total lengths or mean wet weights between the test substance treatment and control groups. Therefore, the lowest observed effect concentration (LOEC) and NOEC in this study were determined to be > 0.094 and 0.094 mg as/L, respectively.

Conclusions

Picolinafen did not result in any toxicity to rainbow trout during 28 days of continuous exposure to water concentrations as high as 0.094 mg as/L. Therefore, the NOEC of AC 900001 to rainbow trout during 28 days of continuous, prolonged exposure is 0.094 mg as/L. The test is valid and reliable. It was conducted according to OECD 204, which it is not considered as adequate test for the assessment of long-term aquatic hazard. It is considered as supplementary information for classification purposes.

11.6.1.2 Study 2

Author: Anonymous

Title: Early Life-Stage test of the Toxicity of AC 900001 to the Rainbow trout (*Oncorhynchus mykiss*)

Date: 1999

Doc ID: ECO 97-310; ABC 44368 , WAT1999-517

Guidelines: U.S. EPA 72-4(a) and OECD 210

GLP: Yes

Validity: Valid

Previous evaluation: In initial DAR (2000)

Materials and methods

A test was conducted to evaluate the toxicity of technical grade AC 900001 (Lot Number CA 14113, 97.8 % pure) to rainbow trout during the early life-stages of development. The test consisted of five AC 900001 exposure groups, a no-treatment control, and a vehicle (dimethylformamide, DMF) blank. Test solutions were prepared and delivered to the test vessels by a proportional diluter system. The following nominal

concentrations of AC 900001 were tested: 0.0 (control), 0.0 (vehicle blank), 5.0, 9.9, 20, 40, and 79 µg as/L (ppb).

The definitive test was initiated with the addition of 25 rainbow trout eggs (approximately two hours of age) into each embryo incubation cup. There were four incubation cups per each test substance treatment and control group, resulting in a total of 100 embryos in each treatment and control at test initiation.

The embryos were observed daily for mortality. After hatching, the embryos were thinned to 15 per replicate (60 per treatment) on test day 24. Survival of the post-hatch fry was monitored until 60 days post-hatch (test termination). At 60 days post-hatch, the blotted wet weight and standard length of each remaining fish was determined.

On test days -2, 0, 7, 11, 12, 14, 21, 28, 35, 42, 56, 63, 70, 77, 83, 90, and 95, composite test solution samples were collected from each treatment and control group and analysed for AC 900001 concentrations. AC 900001 concentrations were determined using a validated HPLC method.

Results and Discussion

The mean measured concentrations of AC 900001 during the 95-day test were 3.1, 6.4, 12, 23, and 42 µg as/L. The mean measured concentrations ranged from 53 to 65 % of the nominal concentrations. AC 900001 residues were not detected in the no-treatment of the vehicle blank (LOD = 0.723 µg/L).

The effect of the various treatments on hatching, survival, standard length and blotted wet weight of rainbow trout is summarised in the table below.

Table 56: Effect of picolinafen on hatching, survival, and growth (standard length and wet weight) of rainbow trout during the Early Life-Stages of development

Treatment	% Hatch	% Survival	Mean Standard Length (mm)	Mean Wet Weight (g)
control	100	100	49.9	1.715
vehicle control	100	95	48.1	1.549
3.1 µg/L	100	93	48.0	1.557
6.4 µg/L	100	95	48.2	1.573
12 µg/L	100	100	45.9*	1.343*
23 µg/L	100	97	44.5*	1.197*
42 µg/L	100	88*	33.8[a]	0.472[a]

*Statistically different ($p \leq 0.05$) from pooled control.
[a]Excluded from growth analyses because of significant survival effects

There was 100 % hatch in all treatments. After 60 days post-hatch, there was a statistically significant reduction in survival in the 42 µg/L treatment. Therefore, the lowest-observed-effect concentration (LOEC) and no-observed effect concentration (NOEC) for survival were 42 and 23 µg/L, respectively.

After 60 days post-hatch, growth, as measured by both mean standard length and blotted wet weights, were significantly reduced at 12 and 23 µg as/L. Therefore, the LOEC and NOEC based on effects on growth were 12 and 6.4 µg as/L, respectively.

Conclusions

Growth, as measured by both standard length and wet weight, was the most sensitive endpoint during the early life-stages of rainbow trout. The LOEC and NOEC values based on this endpoint were 12 and 6.4 µg of AC 900001/L, respectively. The test is valid and reliable. It is relevant for classification purposes.

11.6.2 Chronic toxicity to aquatic invertebrates

11.6.2.1 Study 1

Author:	Barker, C.L.; Ward, G.S. and Hurshman; B.
Title:	Chronic Toxicity of AC 900001 During the complete Life-Cycle of *Daphnia magna* Under Flow-Through Test Conditions
Date:	1998
Doc ID:	ECO 97-164 , WAT1999-535
Guidelines:	U.S. EPA 72-4(b) and OECD 202, Part B
GLP:	Yes
Validity:	Valid

Materials and methods

Groups of forty *Daphnia magna*, less than 24 hours of age, were exposed to technical grade picolinafen (AC 900001, Batch Number 001, 97.8 % pure) for 21 days under flow-through test conditions. The test organisms were equally divided between 4 replicate test vessels. Test solutions were prepared and delivered to the test vessels by a proportional diluter system. A vehicle (acetone) blank was also tested in addition to a no-treatment control group. Nominal test concentrations for the 21-day definitive test were, 0.0 (control), 0.0 (vehicle blank), 5.0, 10, 20, 40, and 80 µg as/L.

The numbers of immobile first generation *Daphnia* in each treatment were recorded throughout the definitive test. Beginning on test day 8, when offspring were first observed, offspring were collected and enumerated every 2 to 3 days. After 21 days of exposure, effects of the test substance on growth (i.e., dry weights and total lengths) were evaluated. The actual exposure concentrations were verified during the test using a validated HPLC method.

Results and Discussion

The mean measured exposure concentrations of AC 900001 during the 21-day test period were: 0.0(control), 0.0 (vehicle blank), 3.97, 7.06, 14.9, 25.8, and 50.9 µg as/L (ppb). The mean measured concentrations ranged from 64 to 79 % of the targeted nominal concentrations.

The effect of the various treatments on survival, reproduction (offspring per adult per reproductive day), total length and dry weights of *Daphnia magna* during 21 days of exposure is summarised in the table below.

Table 57: Effect of picolinafen on survival, reproduction, and growth (total length and dry weight) of Daphnia magna during a complete life-cycle

Treatment[a]	% Survival	Offspring / Adult Reproductive Day	Mean Total Length (mm)	Mean Dry Weight (mg)
control		100	9.14	4.07
vehicle control	97	12.3	4.09	0.85
3.97 µg/L	82	10.0	4.09	0.80
7.06 µg/L	97	9.99	4.05	0.75
14.9 µg/L	82*	6.43*	3.96*	0.67*
25.8 µg/L	25*	7.58*	3.92*	0.62*
50.9 µg/L	47*	0.41*	2.47*	0.086*

[a]Concentrations represent mean measured concentrations of AC 900001.
*Significantly different from controls

After 21 days of exposure, survival was significantly less in all treatments ≥ 14.9 µg as/L in comparison to the pooled controls. Although there was also a statistically significant reduction in survival in the 3.97 µg as/L treatment, this is not considered a test substance-related effect since survival in the 7.06 µg as/L treatment was statistically comparable to the pooled controls. Therefore, the lowest observed effect concentration (LOEC) and the no-observed effect concentration (NOEC) for effects on survival were 14.9 µg as/L and 7.06 µg as/L, respectively. The 21-day LC_{50} was 20.4 µg as/L. Because of the clear effects on survival in the 25.8 µg as/L and 50.9 µg as/L treatments, these groups were excluded from statistical comparisons for sublethal effects.

The number of offspring produced per adult reproductive day was significantly lower in the 14.9 µg/L treatment in comparison to the pooled controls. Therefore, the LOEC and NOEC for effects on reproduction were 14.9 µg as/L and 7.06 µg as/L, respectively.

Both the mean total lengths and mean dry weights were significantly lower in the 14.9 µg as/L treatment in comparison to the pooled controls. Therefore, the LOEC and NOEC for effects on growth were 14.9 µg as/L and 7.06 µg as/L, respectively.

Conclusions

Based on effects on survival, reproduction and growth, the LOEC and NOEC values for Picolinafen during chronic exposure to *Daphnia magna* were 14.9 µg as/L and 7.06 µg as/L, respectively. The test is valid and reliable. It is relevant for classification purposes.

11.6.3 Chronic toxicity to algae or other aquatic plants

Please refer to section 11.5.3. Endpoints used for acute and chronic classification regarding algae and other aquatic plants do not differ and are not repeatedly listed in this section.

11.6.4 Chronic toxicity to other aquatic organisms

Author: Wisk, J.; Barker, C.; England, D.C.; Ward, G.S. and Stewart, S.
Title: Evaluation of the toxicity of AC 900001 to the Sediment Dwelling Larvae of the Midge, *Chironomus riparius*
Date: 1998
Doc ID: ECO 96-310 , WAT1999-526
Guidelines: BBA Draft Guideline "Effects of plant protection products on the sediment-dwelling larvae of *Chironomus repress* in a water-sediment system, and ASTM Guidelines.
GLP: Yes
Validity: Valid
Previous evaluation: In initial DAR (2000)

Materials and methods

A 28 day toxicity test was conducted with larvae of the freshwater midge, *Chironomus riparius* by exposing first instar larvae to ^{14}C-radiolabelled Picolinafen (AC 900001, Lot Number AC 10011-110, 97.8 % radiopurity) in a water/sediment system under static test conditions. The water/sediment system consisted of approximately 200 mL of wet artificial sediment (i.e., 10 % sphagnum peat, 20 % kaolin clay, and 70 % industrial sand) and approximately 1800 mL of hard blended water in 2-L Pyrex glass beakers. The beakers were equipped with mesh cages to capture any emerged adults. Test organisms were added to the beakers approximately 24 hours prior to dosing the systems with different concentrations of ^{14}C-AC 900001. Dosing solutions of ^{14}C-AC 900001 were prepared with acetone as a carrier vehicle, and the test solutions were prepared so that the water concentrations of acetone would not exceed 0.1 mL/L.

Based on the results of two range-finding toxicity tests, the test systems were dosed to provide the following initial water concentrations of AC 900001: 0.038, 0.075, 0.15, 0.30, and 0.60 mg/L. Vehicle blank (0.1 mL acetone/L) and a no-treatment control systems were also prepared. For each treatment and control group, there were eight biological replicates for each treatment and control groups that contained approximately 25 larvae each at test initiation. There were an additional six replicate systems for each treatment concentration and control that did not contain any larvae, and served as analytical replicates.

On exposure days 0 (approximately 2 hours post-dosing), 10, and 28, two of the six analytical replicates were sacrificed and the concentrations of ^{14}C-AC 900001 equivalents in the water, sediment and interstitial water were determined by liquid scintillation counting (LSC). In addition, the water concentration of AC 900001 was confirmed in the highest treatment level on these sampling days using a validated HPLC method.

On exposure days 10 and 28, four of the eight biological replicates were sacrificed and the number of live and dead larvae was determined. Larvae not accounted for were considered dead. Growth of the larvae at day 10 was evaluated by determining larval dry weights. In the four replicates that were not sacrificed until day 28, emergence of adults was evaluated by recording the time to emergence and the total number of emerged adults. The sex of the emerged adults was also determined.

Each of the biological endpoints (i.e., survival, growth at day 10, and adult emergence) was evaluated statistically to determine the lowest observed effect concentration (LOEC) and the NOEC. Results of the study are based on the initial measured water concentrations of ^{14}C-AC 900001 equivalents.

Results and Discussion

On exposure day 0, mean measured water concentrations of ^{14}C-AC 900001 as determined by LSC were 0.043, 0.085, 0.18, 0.48, and 0.69 mg/L, representing 113, 113, 121, 161, and 114 % of the initial nominal water concentrations. Water column concentrations had decreased to 41 to 49 % of the initial nominal concentrations by day 10, and to 26 - 36 % of the initial nominal concentrations by day 28. On day 0, the water column concentration of AC 900001 in the highest concentrations treatment group was determined by HPLC to be 0.53 mg/L, which represented 78 % of the measured concentration of ^{14}C-AC 900001 equivalents as determined by LSC. In water samples from exposure days 10 and 28, no AC 900001 was detected by HPLC analysis, indicating that the test material was degrading in the test systems.

Interstitial water and sediment concentrations of ^{14}C-AC 900001 equivalents increased over the 28-day test period. On day 0, interstitial water concentrations were below the minimum quantifiable limit (MQL) of 0.1 µg/L in the two lowest treatments, were at or below the MQL in the mid-level treatment, and averaged 0.51 and 1.7 µg ^{14}C-AC 900001 equivalents/L in the two highest treatments. Interstitial water concentrations increased by a factor of approximately 100-200X by day 10, with small increases from days 10 to 28. The concentrations of ^{14}C-AC 900001 equivalents/L in the interstitial water never represented more than 1 % of the total ^{14}C-residues in the systems.

Average sediment concentrations in the 5 treatment groups ranged from 0.022 to 0.36 mg ^{14}C-AC 900001 equivalents/kg on day 0, and increased to 0.20 to 3.2 mg ^{14}C-AC 900001 equivalents/kg on day 10. On day

28, concentrations ranged from 0.18 mg ^{14}C-AC 900001 equivalents/kg in the lowest treatment to 2.9 mg ^{14}C-AC 900001 equivalents/kg in the highest treatment. Sediment residues were 2 - 4 % of the total ^{14}C-residues on day 0, 44 - 55 % of the total ^{14}C-residues on day 10, and 51 - 60 % of the total ^{14}C-residues on day 28.

The effect of Picolinafen on midge survival, growth and emergence is summarised in table below.

Table 58: Effect of Picolinafen on the survival, growth and development time of Chironomus riparius

Treatment	Survival		Day 10 Mean Dry Weight	Mean Development Time (Days)
	Day 10	Day 28		
Control	96 %	94 %	1.2 mg	13.6
Vehicle Blank	93 %	99 %	1.4 mg	13.8
0.043 mg/L	92 %	100 %	1.2 mg	13.4
0.085 mg/L	96 %	97 %	1.2 mg	13.0
0.18 mg/L	95 %	93 %	1.1 mg	13.5
0.48 mg/L	98 %	99 %	1.1 mg*	14.1
0.69 mg/L	92 %	80 %*	1.0 mg*	16.0*

*Significantly different from the controls

In comparison to the controls, there was a statistically significant difference in survival, as measured by adult emergence, in the highest treatment group. Therefore, the 28-day LC_{50} was > 0.69 mg ^{14}C-AC 900001 equivalents/L and the NOEC for survival was 0.48 mg ^{14}C-AC 900001 equivalents/L.

There was a statistically significant reduction in the dry weights on the midge in the two highest treatment groups (0.48 and 0.69 mg ^{14}C-AC 900001 equivalents/L) in comparison to the vehicle blank. Therefore, the NOEC for effects on day 10 dry weights was 0.18 mg ^{14}C-AC 900001 equivalents/L.

There was a statistical difference in time to emergence between the 0.69 mg ^{14}C-AC 900001 equivalents/L treatment group and the pooled controls. Therefore, the NOEC for effects on adult emergence was 0.48 mg ^{14}C-AC 900001 equivalents/L.

Conclusions

The most sensitive endpoint observed in the study was effects on larval dry weights at day 10.

The NOEC based on this endpoint and initial measured concentrations of ^{14}C-AC 900001 equivalents was 0.18 mg ^{14}C-AC 900001 equivalents/L. Adult emergence was affected at 0.69 mg ^{14}C-AC 900001 equivalents/L, the highest concentration tested. Therefore, the 28-day NOEC was 0.48 mg ^{14}C-AC 900001 equivalents/L. The study is valid and reliable. It is considered relevant for classification purposes.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Picolinafen produces acute $L(E)C_{50}$ values in concentrations > 0.0001 ≤ 0.001 mg/L for algae, > 0.01 ≤ 0.1 mg/L for aquatic plants, > 0.1 ≤ 1 mg/L for crustaceans and for fish.

According to the criteria of the CLP Regulation, a substance is classified for aquatic acute toxicity if in an aquatic acute toxicity study, an $L(E)C_{50}$ of ≤ 1 mg/l is obtained for any of the three trophic levels fish, invertebrates and algae/aquatic plants.

The lowest $L(E)C_{50}$ obtained for Picolinafen are 0.00038, 0.057, > 0.45 and > 0.68 mg/L in algae, aquatic plants, invertebrates and fish, respectively. Picolinafen therefore fulfils the criteria for classification as Aquatic Acute Cat. 1.

An M-factor of 1000 for acute toxicity is proposed based on E_rC_{50} value of 0.00038 mg/L in algae ($0.0001 < L(E)C_{50} \leq 0.001$ mg/L).

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

Chronic NOEC values in concentrations > 0.00001 ≤ 0.0001 mg/L for algae and > 0.001 ≤ 0.01 mg/L for aquatic plants, invertebrates and fish were determined. The lowest NOECs per organisms group were 0.000098 mg/L for algae (*Pseudokirchnerialla subcapitata*), 0.0072 for aquatic plants (*Lemna gibba*), 0.00706 mg/L for aquatic invertebrates (*Daphnia magna*) and 0.0064 mg/L for fish (*Oncorhynchus mykiss*).

Based on a ready biodegradation test (OECD 301 D), picolinafen is not considered readily biodegradable (7 % biodegradation in 28 days). According to hydrolysis test (OECD 111), picolinafen is hydrolytically stable in solutions at pH 4 to 9. Studies on direct photolysis in water show that direct photodegradation in aqueous systems is insignificant under environmental conditions. In water/sediment systems picolinafen was immediately removed to the sediment phase and degraded quickly both in the water as well as in the sediment phase. Degradation of picolinafen in the total water/ sediment systems followed SFO kinetics with DT_{50} values of 5.4 days and DT_{90} values of 17.8 days. The main metabolite CL 153815, which reached maxima in the total systems of > 30 % and > 90 % after 100 d, degraded itself with DT_{50} values of 96 d and 578 d (SFO kinetic) respectively. Mineralisation to carbon dioxide with 2.5 % after 100 d in both systems indicates that the CLP criteria of ultimate degradation of > 70 % within 28 days is not fulfilled for picolinafen. Therefore, picolinafen is considered being not rapidly degradable according to the CLP criteria.

Picolinafen has a log Kow of 5.4. The experimentally derived kinetic BCF of 617 for Picolinafen related to parent, whole fish and lipid normalised is higher than the trigger of 500 (criterion for bioaccumulation potential conform Regulation EC 1272/2008).

The assignment of a hazard category depends on the NOEC value and whether the substance is rapidly degradable or not. According to the criteria of the 2nd ATP to the CLP Regulation, when NOEC values are available for all trophic levels, a non-rapidly degradable substance is classified for aquatic chronic hazards if a NOEC or EC_{10} of ≤ 0.1 mg/L is obtained in a long-term aquatic toxicity study.

The lowest NOE_rC is 0.000098 mg/L obtained for algae. Picolinafen therefore fulfils criteria for classification as Aquatic Chronic Cat. 1.

An M-factor of 1000 for chronic toxicity is proposed based on the NOE_rC value of 0.000098 mg/L for algae. ($0.00001 < NOEC \leq 0.0001$ mg/L for non-rapidly degradable substances).

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Picolinafen fulfils the criteria for classification as Aquatic Acute 1 with an M-factor of 1000.

Picolinafen fulfils the criteria for classification as Aquatic Chronic 1 with an M-factor of 1000.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Overall, the DS concluded that picolinafen is 'not rapidly degradable', has a potential for bioaccumulation and proposed classification based on aquatic acute and chronic toxicity to algae:

Aquatic Acute 1 with an M-factor of 1000 based on the lowest mean measured 72-hour E_rC_{50} value of 0.00038 mg/L for *Pseudokirchneriella subcapitata*; and

Aquatic Chronic 1 with an M-factor of 1000 based on the lowest mean measured 72-hour NOE_rC of 0.000098 mg/L for *Pseudokirchneriella subcapitata*.

Degradation

Based on a ready biodegradation test (OECD TG 301D, GLP), picolinafen is not considered readily biodegradable (7% biodegradation in 28 days) (Leberts, 1996).

According to the available hydrolysis test (OECD TG 111, GLP), picolinafen is hydrolytically stable in solutions at pH 4, 7 and 9 at 50 ± 0.1 °C (Schlüter, 1997).

Studies on direct photolysis (OECD TG 316, GLP) in water show that direct photodegradation in aqueous systems is insignificant under environmental conditions with DT_{50} values of 54 and 88.8 days at pH 7 (McLaughin, 2012).

In the study "Determination of the Direct Phototransformation in Buffered Medium at pH 7" (OECD draft TG "Phototransformation of Chemicals in Water"), picolinafen was slowly degraded by photolysis (Knoch and Yan, 1998) with a DT_{50} of 290.7 hours (12.1 days) using an artificial light source (relative intensity: 2.34 sun hours per instrument hour) under laboratory conditions. No environmental half-life calculation was performed in this study.

In the river and pond water/sediment systems (SETAC Guideline, OECD Draft Proposal, GLP), 40.1% and 70.6%, respectively, of picolinafen was immediately removed to the sediment phase and the remaining degraded quickly both in the water as well as in the sediment phase (Yan, 1999, a kinetics assessment Mamouni and Jarvis, 2012). Degradation of picolinafen in the total water/ sediment systems followed SFO kinetics with DT_{50} values of 5.4 days and DT_{90} values of 17.8 days. The main metabolite CL 153815, which reached maxima in the total systems of > 30% and > 90% after 100 days, degraded with DT_{50} values of 96d and 578d (SFO kinetic), respectively. Mineralisation to carbon dioxide at 2.5% after 100d in both systems indicates that the CLP criteria of ultimate degradation of > 70% within 28 days are not fulfilled for picolinafen.

Overall, due to the results summarised above, the DS concluded that degradation information does not show that picolinafen is ultimately degraded to > 70% within 28 days (equivalent to a half-life of less than 16 days) or being transformed to non-classifiable products. Therefore, picolinafen was considered by the DS as not being rapidly degradable according to the CLP criteria.

Aquatic Bioaccumulation

A flow-through study (OECD TG 305E, GLP) on bluegill sunfish (*Lepomis macrochirus*) was conducted with picolinafen with nominal concentrations of 2 and 20 ppb for a period of 28 days followed by a 14 day period of depuration in fresh water. Picolinafen was prepared for the study by isotopic dilution of [pyridine-2,6-^{14}C]picolinafen and by isotopic dilution of [p-fluoroanilineU-^{14}C]picolinafen (Anonymous 22, 1998).

For [pyridine-2,6-^{14}C]picolinafen, the whole fish lipid normalised steady-state bioconcentration factor was calculated to be 589 L/kg for the treatment level of 2 ppb and a non-lipid normalised steady-state bioconcentration factor was calculated to be 640 L/kg for the treatment level of 20 ppb.

For [p-fluoroanilineU-^{14}C]picolinafen, the whole fish lipid normalised steady-state bioconcentration factors were calculated to be 438 and 561 L/kg for the treatment level of 2 and 20 ppb, respectively.

A kinetic bioconcentration factor equal to 617 L/kg for whole fish based on *Lepomis macrochirus* and normalised to 5% lipid content was derived for the treatment level of 20 ppb of [pyridine-2,6-^{14}C]-picolinafen. The time for 50% depuration was 1.2 days and depuration was > 95% after 14 days.

The experimentally derived kinetic BCF of 617 L/kg for picolinafen related to whole fish and lipid normalised is above the CLP criteria trigger value of ≥ 500. A Log K_{OW} of 5.4 at 25 °C (almost the same in different buffered media at pH 5, 7, 9) also meets the CLP criteria trigger value of ≥ 4 indicating a potential for bioaccumulation. Therefore, the DS considers picolinafen to have a potential to bioaccumulate.

Aquatic Toxicity

The aquatic toxicity test results for picolinafen from available acute and chronic studies for all trophic levels are summarised in the following table and sections. Acute and chronic aquatic toxicity data for picolinafen are available for fish, invertebrates, algae and aquatic plants. Algae are the most acutely and chronically sensitive trophic level. Provided studies were considered acceptable and reliable by the DS.

The studies for picolinafen degradants (CL 153815, CL 7693) with fish, invertebrates and algae are useful indicators that the degradants are significantly less toxic than the parent substance (picolinafen). Therefore, metabolites are not considered more toxic than the parent substance and data on metabolites' toxicity have not been included in the table below, although they have been shortly presented in the text in this section.

Aquatic Acute toxicity

***Table**: Aquatic Acute toxicity studies*

Test organism	Test method / reliability	Short-term result (endpoint)	Reference / Test item
Fish			
Rainbow trout *(Oncorhynchus mykiss)*	OECD TG 203, GLP / 1	96h LC_{50} > 0.68 mg/L (mm)	Anonymous 23 (1998) / picolinafen (97.8%)
Bluegill sunfish *(Lepomis macrochirus)*	OECD TG 203, GLP / 1	96h LC_{50} > 0.57 mg/L (mm)	Anonymous 24 (1988) / picolinafen (97.8%)

Aquatic invertebrates			
Water flea *(Daphnia magna)*	OECD TG 202, GLP / 1	48h EC_{50} > 0.45 mg/L (mm)	Wisk (1998) / picolinafen (97.8%)
Algae / other aquatic plants			
Freshwater green alga *(Pseudokirchneriella subcapitata)*	OECD TG 201, GLP / 1	**72h E_rC_{50} = 0.00038 mg/L (mm)** 72h E_bC_{50} = 0.00018 mg/L (mm)	Wisk (1998) / picolinafen (97.8%)
Freshwater cyanophyte (blue-green alga) *Anabaena flos-aquae*	OECD TG 201, GLP / 3	120h E_rC_{50} > 0.00039 mg/L (mm) 120h E_bC_{50} = 0.00034 mg/L (mm)	Barker *et al*. (1998) / picolinafen (97.8%)
Freshwater green alga *(Pseudokirchneriella subcapitata)*	OECD TG 201, no-GLP / 1 (supplementary)	72h E_yC_{50} = 0.00017 mg/L (nom)	Barker (1999) / picolinafen (97.8%)
Duckweed *(Lemna gibba)*	American Society for Testing and Materials (1990). Standard Guide for Conducting Static Toxicity Tests with Lemna gibba G3, GLP / 2	72h E_rC_{50} = 0.057 mg/L (initial mm) 72h E_bC_{50} = 0.08 mg/L (initial mm)	Barker (1998) / picolinafen (97.8%)

mm: mean measured concentration, nom: nominal concentration

numbers 1, 2, 3 in the reliability column refer to Klimisch scores

Two studies have been submitted on the acute toxicity of picolinafen in fish. The reported 96-hour LC_{50} values of picolinafen in both studies with fish were in the same range and vary between > 0.1 - < 1 mg/L based on mean measured concentrations. The reported toxicity data of metabolites CL 153815 and CL 7693 were > 100 mg/L and 19.9 mg/L, respectively, based on mean measured concentrations.

One study has been submitted on the acute toxicity of picolinafen in invertebrates. The reported 48-hour EC_{50} value of picolinafen was > 0.45 mg/L based on mean measured concentrations. The reported toxicity data of metabolites CL 153815 and CL 7693 were > 98 mg/L and 0.254 mg/L, respectively, based on mean measured concentration.

Three studies have been submitted on the acute toxicity of picolinafen in algae. However, the study with *Anabaena flos-aquae* (Barker *et al*., 1998) was considered by the DS as not reliable due to the following shortcomings: 1. the mean coefficient of variation for section-by-section specific growth rates in the control cultures exceeds the validity criterion. 2. The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures exceeds the validity criterion. 3. The initial cell numbers were only detected in control replicates but not for treated vessels. Thus, this study was considered not reliable.

According to the reported reliable studies, 72-hour E_rC_{50} and E_bC_{50} values of picolinafen were in the same range and fell between > 0.0001 and < 0.001 mg/L, based on mean measured concentrations. However, reported E_yC_{50} values of picolinafen that fell in the same range were based on nominal concentrations. The reported toxicity data for metabolites CL 153815 and CL 7693 vary between > 1 - < 100 mg/L, based on mean measured concentrations.

One study was submitted on the acute toxicity in aquatic macrophytes (*Lemna gibba*). The reported 72-hour E_rC_{50} and E_bC_{50} values were in the same range and varied between > 0.01

- < 0.1 mg/L, based on initial mean measured concentrations.

Overall, the DS proposed to classify picolinafen as Aquatic Acute 1 based on 72-hour E_rC_{50} for *Pseudokirchneriella subcapitata* of 0.00038 mg/L based on mean measured concentration. As this acute toxicity value falls within the 0.0001 < L(E)C_{50} ≤ 0.001 mg/L range, the acute M-factor proposed by the DS is 1000.

Aquatic Chronic toxicity

***Table**: Aquatic Chronic toxicity studies*

Test organism	Test method / reliability	Long-term result (endpoint)	Reference / Test item
Fish			
Rainbow trout *(Oncorhynchus mykiss)*	OECD TG 204, GLP / 1 (considered only as supplementary information)	28d NOEC = 0.094 mg/L (mm)	Anonymous 27 (1999) / picolinafen (97.8%)
Rainbow trout *(Oncorhynchus mykiss)*	OECD TG 210, GLP / 1	95d NOEC = 0.0064 mg/L (mm)	Anonymous 28 (1999) / picolinafen (97.8%)
Aquatic invertebrates			
Water flea *(Daphnia magna)*	OECD TG 202 (Part B), GLP / 1	21d NOEC = 0.00706 mg/L (mm)	Barker (1998) / picolinafen (97.8%)
Algae /other aquatic plants			
Freshwater green alga *(Pseudokirchneriella subcapitata)*	OECD TG 201, GLP / 1	**72h NOE_rC = 0.000098 mg/L (mm)**	Wisk (1998) / picolinafen (97.8%)
Freshwater cyanophyte (blue-green alga) *Anabaena flos-aquae*	OECD 201, GLP / 3	120h NOE_rC = 0.000063 mg/L (mm)	Barker *et al*. (1998) / picolinafen (97.8%)
Duckweed *(Lemna gibba)*	American Society for Testing and Materials (1990). Standard Guide for Conducting Static Toxicity Tests with Lemna gibba G3, GLP / 2	72h NOE_rC = 0.0072 mg/L (initial mm)	Barker (1998) / picolinafen (97.8%)
Sediment dwelling organisms			
Midge larvae *(Chironomus riparius)*	BBA Draft Guideline, ASTM Guidelines, GLP / 1	10d NOEC = 0.18 mg/L (mm)	Wisk (1998) / Picolinafen (97.8%)

mm: mean measured concentration, nom: nominal concentration

numbers 1, 2, 3 in the reliability column refer to Klimisch scores

Two studies were submitted on the chronic toxicity of picolinafen in fish. However, one study was conducted according to OECD TG 204, which it is not considered as an adequate test for the assessment of long-term aquatic hazard. Therefore, this study was considered only as supplementary information. For the other valid and acceptable study, the reported 95-day NOEC value of picolinafen was 0.0064 mg/L, based on mean measured concentrations.

One study was submitted on the chronic toxicity of picolinafen in invertebrates. The reported

21-day NOEC value of picolinafen was 0.00706 mg/L, based on mean measured concentrations.

Two studies were submitted on the chronic toxicity of picolinafen in algae. However, the study with *Anabaena flos-aquae* (Barker *et al*., 1998) was considered not reliable based on the reasons pointed out in the acute toxicity section. The reported 72-hour NOE_rC value from the reliable study with *Pseudokirchneriella subcapitata* (Wisk, 1998) was 0.000098 mg/L, based on mean measured concentrations.

One study was submitted on the chronic toxicity of picolinafen in aquatic macrophytes (*Lemna gibba*). The reported 72-hour NOE_rC value was 0.0072 mg/L, based on initial mean measured concentrations.

One study on the chronic toxicity of picolinafen in midges (*Chironomus riparius*) in a water/sediment system was submitted. The reported 10-day NOEC value was 0.18 mg/L, based on mean measured concentrations.

Overall, the DS proposed to classify picolinafen as Aquatic Chronic 1 based on 72-hour NOE_rC for *Pseudokirchneriella subcapitata* of 0.000098 mg/L, based on mean measured concentrations. Picolinafen is considered to be not rapidly degradable and chronic toxicity value falls within the 0.00001 < NOEC ≤ 0.0001 mg/L range, thus the chronic M-factor proposed by the DS is 1000.

Comments received during consultation

One MSCAs submitted comment agreeing with the proposed classification by the DS without any remarks.

Assessment and comparison with the classification criteria

Degradation

A ready biodegradability test (OECD TG 301D, GLP) shows that 7% biodegradation after 28 days of picolinafen was observed. Therefore, picolinafen is considered not readily biodegradable.

The results of a hydrolysis study (OECD TG 111, GLP) showed that picolinafen is hydrolytically stable in solutions at pH 4, 7 and 9 at 50 ºC over a period of 5 days.

A photodegradation study in sterile water at pH 7 at 25ºC (OECD TG 316, GLP) with DT_{50} = 54 – 88.8 days shows that direct photodegradation in aqueous systems is insignificant under environmental conditions. Determination of the Direct Phototransformation in Buffered Medium at pH 7 (OECD Draft TG: "Phototransformation of Chemicals in Water", 1992, GLP) indicated that picolinafen was slowly photolysed under the test conditions with DT_{50} 290.7 hours (12.1 days) using the artificial light source with intensity 2.34 sun hours per instrument hour. No environmental half-life calculation was performed in this study.

Two water/sediment systems (river and pond) according to SETAC Guideline, OECD Draft Proposal, GLP were investigated in a flow-through test system using ^{14}C-labelled picolinafen and metabolite ^{14}C-CL 153815. A kinetics assessment was performed as well in accordance with FOCUS degradation kinetics guidance. In the river and pond water/sediment systems (SETAC Guideline, OECD Draft Proposal, GLP), 40.1% and 70.6% of picolinafen was

immediately removed to the sediment phase, respectively, and the remaining test substance degraded quickly both in the water as well as in the sediment phase. Registered DT_{50} and DT_{90} values of picolinafen for the dissipation from water phase were DT_{50} 4.02 (river) and 1.89 (pond) days (DT_{90}s respectively 13.35 and 6.29 days). Degradation in the total system following SFO kinetics were DT_{50} 5.36 (river) and 5.34 (pond) days (DT_{90}s respectively 17.79 and 17.74 days). The main metabolite CL 153815, which reached maxima in the total systems of 31.7 – 32.2% and 94.5 – 92.4% after 100 d, degraded with DT_{50} values of 96 days and 578 days (SFO kinetic) respectively. The maximum carbon dioxide in both systems was 2.7% AR after 100 days, indicating minimal mineralisation of picolinafen.

Overall, due to the results summarised above, RAC agrees with the assessment of the DS that picolinafen is not ultimately degraded to > 70% within 28 days (equivalent to a half-life < 16 days), or rapidly transformed to non-classifiable products. Consequently, RAC agrees that picolinafen should be considered not rapidly degradable under the CLP regulation.

Aquatic Bioaccumulation

In the available experimental study to determine the bioconcentration potential, the determined whole fish BCF value of 617 L/kg for picolinafen (kinetic BCF lipid normalised and growth corrected) is above the CLP criteria trigger value of ≥ 500. The derived Log K_{OW} value of 5.4 at 25 °C (not dependent on pH) also meets the CLP criteria trigger value for indication of bioaccumulation (Log K_{OW} ≥ 4).

Therefore, RAC agrees with the DS that picolinafen is bioaccumulative in the aquatic environment, according to the CLP criteria.

Aquatic Toxicity

RAC notes that there are reliable acute and chronic aquatic toxicity data for all trophic levels. The most acutely and chronically sensitive trophic group is algae. The metabolites CL 153815 and CL 7693 are significantly less toxic than the parent substance so they are not to be considered relevant for classification. RAC agrees that the study with algae *Anabaena flos-aquae* (Barker *et al*., 1998) is considered not reliable as the validation criteria are not met.

Consequently, RAC agrees that the lowest acute endpoint for aquatic acute classification is the 72-hour E_rC_{50} value for *Pseudokirchneriella subcapitata* of 0.00038 mg/L, based on mean measured concentrations. The lowest chronic endpoint for aquatic chronic classification purpose is 72-hour NOE_rC for *Pseudokirchneriella subcapitata* of 0.000098 mg/L, based on mean measured concentrations.

Conclusion on classification

Picolinafen is considered not rapidly degradable and fulfils the criteria for bioaccumulation. Based on the available and reliable information, RAC agrees with the DS that picolinafen warrants classification as:

Aquatic Acute 1 based on E_rC_{50} = 0.00038 mg/L for *Pseudokirchneriella subcapitata.* As this acute toxicity value falls within the 0.0001 < $L(E)C_{50}$ ≤ 0.001 mg/L range, the **acute M-factor is 1000**.

Aquatic Chronic 1 based on NOE_rC = 0.000098 mg/L for *Pseudokirchneriella subcapitata*. As this chronic toxicity value falls within the 0.00001 < NOEC ≤ 0.0001 mg/L range, the **chronic M-factor is 1000.**

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13 ANNEXES

A Risk Assessment Report (Volume 3 - B6) is publicly available (http://registerofquestions.efsa.europa.eu/roqFrontend/outputLoader?output=ON-4279).

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