Committee for Risk Assessment

RAC

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-[(*a*,*a*,*a*-trifluoro-*m*-tolyl)oxy]picolinanilide

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

CLH-O-0000007040-89-01/F

Adopted

16 September 2021

16 September 2021

CLH-O-0000007040-89-01/F

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

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

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

EC Number: **-**

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

The proposal was submitted by **Germany** and received by RAC on **4 September 2020.**

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

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **5 October 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **4 December 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

Co-Rapporteur, appointed by RAC: **Žilvinas Užomeckas**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 September 2021** by **consensus**.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	picolinafen (ISO); *N*-(4-fluorophenyl)-6-[3-(trifluoromethyl)phenoxy]pyridine-2-carboxamide; 4′-fluoro-6-[(α,α,α-trifluoro-*m*-tolyl)oxy]picolinanilide	-	137641-05-5	STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H373 (blood, thyroid) H400 H410	GHS08 GHS09 Wng	H373 (blood, thyroid) H410		M = 1000 M = 1000	
RAC opinion	TBD	picolinafen (ISO); *N*-(4-fluorophenyl)-6-[3-(trifluoromethyl)phenoxy]pyridine-2-carboxamide; 4′-fluoro-6-[(α,α,α-trifluoro-*m*-tolyl)oxy]picolinanilide	-	137641-05-5	STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H373 (blood system, thyroid) H400 H410	GHS08 GHS09 Wng	H373 (blood system, thyroid) H410		M = 1000 M = 1000	
Resulting Annex VI entry if agreed by COM	TBD	picolinafen (ISO); *N*-(4-fluorophenyl)-6-[3-(trifluoromethyl)phenoxy]pyridine-2-carboxamide; 4′-fluoro-6-[(α,α,α-trifluoro-*m*-tolyl)oxy]picolinanilide	-	137641-05-5	STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H373 (blood system, thyroid) H400 H410	GHS08 GHS09 Wng	H373 (blood system, thyroid) H410		M = 1000 M = 1000	

GROUNDS FOR ADOPTION OF THE OPINION

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.

HUMAN HEALTH HAZARD EVALUATION

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.

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.

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.

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.

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.

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.

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.

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.

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 (unilateral)</td><td>0</td><td>1</td><td>1</td><td>1</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:</td><td>5</td><td>5</td><td>5</td><td>8</td></tr>
</table>
</td>
<td>Anonymous 18, 1999</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></td><td></td><td>
<table>
<tr><td>benign neoplasm (unilateral)</td><td>(7.7%)</td><td>(11.9%)</td><td>(11.6%)</td><td>(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.

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.

2-generation study

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).

Repeated-dose toxicity studies

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.

ENVIRONMENTAL HAZARD EVALUATION

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

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 $0.00001 < NOEC \leq 0.0001$ 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.**

Additional references

Muller *et al.*, 2006. Hazard classification of chemicals inducing haemolytic anaemia: An EU regulatory perspective. Regulatory Toxicology and Pharmacology, vol.45 (3), pp 229-241, August 2006.

EFSA, RAR (2015): Renewal Assessment Report. Picolinafen. Vol. 3, section B.6. Toxicology and Metabolism. Revised 23 April 2015

Consistent target organ/ organ system designation with specific target organ toxicity (STOT) classification – blood. ECHA, June 2021

ANNEXES:

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

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