Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Isoproturon (ISO);
3-(4-isopropylphenyl)-1,1-dimethylurea

EC Number: 251-835-4
CAS Number: 34123-59-6

CLH-O-0000001412-86-115/F

Adopted

3 June 2016

3 June 2016

CLH-O-0000001412-86-115/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: **isoproturon (ISO); 3-(4-isopropylphenyl)-1,1-dimethylurea**

EC Number: **251-835-4**

CAS Number: **34123-59-6**

The proposal was submitted by **Germany** and received by RAC on **4 November 2015**.

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 **9 December 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **25 January 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Stine Husa**

Co-Rapporteur, appointed by RAC: **João Carvalho**

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 **3 June 2016** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	006-044-00-7	isoproturon (ISO); 3-(4-isopropylphenyl)-1,1-dimethylurea	251-835-4	34123-59-6	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M=10	
Dossier submitters proposal	006-044-00-7	isoproturon (ISO); 3-(4-isopropylphenyl)-1,1-dimethylurea	251-835-4	34123-59-6	**Retain:** Aquatic Acute 1 Aquatic Chronic1 **Add:** Repr. 2 STOT RE 2	**Retain:** H400 H410 **Add:** H361f H373 (oral, blood)	**Retain:** GHS08 GHS09 Wng	**Retain:** H410 **Add:** H361f H373 (oral, blood)		**Retain:** M=10 **Add:** M=10	
RAC opinion	006-044-00-7	isoproturon (ISO); 3-(4-isopropylphenyl)-1,1-dimethylurea	251-835-4	34123-59-6	**Add:** STOT RE 2 **Retain:** Aquatic Acute 1 Aquatic Chronic 1	**Add:** H373 (blood) **Retain:** H400 H410	**Retain:** GHS08 GHS09 Wng	**Add:** H373 (blood) **Retain:** H410		**Retain:** M=10 **Add:** M=10	
Resulting Annex VI entry if agreed by COM	006-044-00-7	isoproturon (ISO); 3-(4-isopropylphenyl)-1,1-dimethylurea	251-835-4	34123-59-6	Carc. 2 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H373 (blood) H400 H410	GHS08 GHS09 Wng	H351 H373 (blood) H410		M=10 M=10	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Isoproturon has an existing entry in Annex VI of CLP as Carc. 2 (H351). The DS did not address carcinogenicity in the CLH dossier since it is already harmonised. The Public Consultation (PC) of the CLH proposal was opened for comments from 9 December 2015 until 25 January 2016. Concerned parties were invited to comment on the hazard classes reproductive toxicity, specific target organ toxicity (repeated exposure) and hazardous to the aquatic environment (M-factors) only. This is in line with the agreement made at CARACAL-13 between ECHA, the Commission and Member States (Doc. CA/17/2013). During PC however, the applicant submitted a comment and two attachments arguing against the existing Carc. 2 (H351) classification.

During the Peer Review Meeting 125 (25 – 27 February 2015), EFSA as well as the Experts agreed that the non-relevance for humans of mode of action for liver tumours in the rat was not clearly demonstrated by the applicant on the basis of the new mechanistic data provided. In particular, proliferation was observed in wild type rats, while enzyme induction was observed in both wild and CAR knock-out rats and this would not support the claim that the activation of the CAR is the only mode of action. Further indications for another mode of action that might be relevant for humans (i.e. PXR activation) are also available. EFSA and the experts considered that the harmonised classification and labelling for carcinogenicity (Carc. 2; H351) is still appropriate.

The Rapporteur member state (RMS) (Germany) summarised the above in the revised Renewal Assessment Report (published as a revised version on 8 April 2015), together with theirreview of the new mechanistic data and further details concerning pituitary and mammary gland tumours in rats. However, no change to the existing Carc. 2 (H351) classification and labelling of isoproturon was proposed by the RMS.

As carcinogenicity was not considered as part of the Annex XV CLH proposal by the DS, RAC was unable to provide an opinion on the carcinogenicity of isoproturon.

HUMAN HEALTH HAZARD EVALUATION

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

Summary of the Dossier Submitter's proposal

The DS evaluated 11 repeated dose toxicity studies conducted with isoproturon by the oral (dietary) route: 3 subacute (28-day) studies in rats, mice and dogs, 8 subchronic (90-day) studies in rats, dogs and monkeys. In addition 3 studies (rats, 14-day and 90-day) by the inhalation route and 4 studies (rats and rabbits, 21-day and 90-day) by the dermal route were evaluated. The DS considered the liver and the blood to be the main target organs.

Liver effects were confined to organ weight increases in the rat, dog and monkey beginning at dietary concentrations of 500 ppm (12.5 mg/kg bw/d) in dogs. At higher dose levels histopathological changes in the liver were concomitant with haemolytic anaemia. The findings in the liver (increased relative and absolute weight, bile duct proliferation, degeneration of hepatocytes, basophilic foci, increased hemosiderin in the liver) were associated with increased

enzyme activities (AP, ALT, AST) and reductions in total protein or albumin in blood. There was evidence that the effects seen were reversible. The hepatocellular findings were not considered significant or severe by the DS, and were not observed at dose levels below the guidance values warranting classification for STOT RE 2 according to CLP criteria. Liver toxicity was not observed after dermal and inhalation exposure.

Anaemia was observed at or above dietary concentrations of approximately 800 ppm (62 mg/kg bw/d) in rats, 500 ppm (12.5 mg/kg bw/d) in dogs and 150 mg/kg bw/d in monkeys. The severity of the anaemia increased dose-dependently and was associated with Heinz bodies, methemoglobinaemia, hyperplastic bone marrow, extramedullary hematopoiesis and increased hemosiderin in liver, kidneys and bone marrow, indicating toxic haemolytic anaemia.

The DS concluded that the haematotoxicity (decrease in Hb by 20 % along with haemosiderin deposition in reticular cells and Kupffer cells of the liver), reported in rats in the oral 90-day repeated dose studies (below 100 mg/kg bw/d) and at the mid and high dose levels at the end of the oral 90-day dog study (essentially at ca 4 and 12.5/20 mg/kg bw/d, respectively), are sufficient to fulfil the criteria for severity to warrant classification for STOT RE 2 (blood) (oral). Further the DS concluded that the repeated dose toxicity studies by inhalation and dermal routes did not demonstrate any haematotoxicity that would justify classification.

Comments received during public consultation

Two Member state competent authorities (MSCAs) supported the proposed classification for STOT RE 2 (blood) (oral). Industry did not specifically comment on the STOT RE 2 classification but recognised that haemolytic anaemia is a clear and consistent toxicological effect of isoproturon.

Assessment and comparison with the classification criteria

As described by the DS, studies with repeated exposure in rats, mice, rabbits, dogs and monkeys are available. Briefly, the findings from these studies considered relevant by RAC are described below.

Rat

Rats, 28-day, oral (diet) (Scholz and Weigand, 1973)

Rats (SPF-Wistar) were exposed to isoproturon at dose levels of 0, 500, 1250, 3200, 8000 and 20000 ppm in a range-finding-test. The corresponding achieved doses were 0/0, 42.8/43.4, 105.3/105.6, 258.8/254.6, 538.5/523.1 and 911.2/602.2 mg/kg bw/d in males/females. In the top treatment group all rats died between days 6 and 18. Body weight gains were statistically significant reduced in all treatment groups except for the 500 ppm group (females). The relative liver weights were statistically increased in males receiving 8000 or 3200 ppm and females receiving 8000, 3200 or 1250 ppm immediately following the treatment period. This effect was not evident after 14 days of recovery.

Rats, 90-day, oral (diet) (Leuschner et al., 1973)

Rats were exposed to isoproturon at dose levels of 0, 80, 400, 2000 and 10000/20000 ppm (increased after 6 weeks) of isoproturon in diet for a 13 weeks. The corresponding achieved doses were 0/0, 6.9/7.0, 36.6/34.4, 171/175, 990/1032 and 1491/1557 mg/kg bw/d in males/females. Body weight gain in the group receiving 2000 ppm was depressed from week 2 onwards by up to 8.5 % in males and by up to 10.1 % in females resulting in a statistically significant lower mean body weight in both sexes in weeks 6 and 13. No other clinical signs were seen at doses up to 2000 ppm. Body weight gain of rats fed 10000 ppm during the first 6 weeks

was severely depressed and animals lost weight after the dose had been increased to 20000 ppm (48-50% reduction after 13 weeks). Feed consumption were closely related to body weight gain. No other clinical signs were seen in rats treated at 10000 ppm for 6 weeks. Following the elevation of the top dose treatment level to 20000 ppm the rats became progressively quieter and more apathetic. One male and 2 females died at this high dose level in the 13th week. Rats receiving 10000/20000 ppm showed a statistically significant increase (170%) in the methaemoglobin (Met-Hb) content and in the number of Heinz bodies at the end of the dosage period.

Rats, 90-day, oral (gavage) (Bhide, 1984)

Rats were exposed to isoproturon at dose levels of 0, 85, 250 and 750 mg/kg bw/d for 90 days. In addition, a group of rats was treated with 250 mg/kg bw/d for 90 days and observed for reversibility of toxic effects for a post-treatment period of 30 days. Feed intake and body weight gain was reduced in the highest dose groups and haematological changes (including reduction in Hb, PCV and RBC, increase in methaemoglobin content and reticulocyte and presence of Heinz bodies) was observed starting at 250 mg/kg bw/d. No mortality was observed at any dose level.

Rats, 90-day, oral (diet) (Dickhaus and Heisler, 1987)

Rats (Wistar) were exposed to isoproturon at dose levels of 0, 400, 1500 and 5000 ppm of isoproturon for a 90-day period. Corresponding doses of 0, 20-40, 75-150, and 250-500 mg/kg bw/d were calculated based on a conversion factor of 0.05-0.1. Decreased body weight gains and feed consumption and increased weights of liver was observed in the highest dose groups.

Rats, 90-day, oral (diet) (Bhide, 1990)

Rats (Wistar) were exposed to isoproturon at dose levels of 0, 400, 1200 and 2400 ppm of isoproturon for a period of 90 days. Corresponding doses of 0, 20-40, 60-120, and 120-240 mg/kg bw/d were calculated based on a conversion factor of 0.05-0.1. In the highest dose groups intermittent diarrhoea, reduction in body weight gain, haemosiderosis and hyperplasia of the bone marrow cells was observed. Hb, PCV and RBC were decreased, Heinz bodies were seen and MetHb and liver weight was increased at 2400 ppm.

Rats, 90-day oral (diet) (Wragg et al., 1991)

Rats (Sprague-Dawley CD strain) were exposed to isoproturon at dose levels of 0, 80, 800 and 8000 ppm for 90 days. 80 ppm corresponded to 5.6 mg/kg bw/d. Reduced bodyweight gain and feed consumption, a reduction in RBC, Hb and Hk, together with an increase in the MCV, elevated reticulocyte count and increased relative MetHb was seen in high dose animals. These animals also showed an increase in prothrombin time. Total protein, albumin, glucose and urea were reduced in high dose animals. Six males from the high dose group showed small seminal vesicles and a small prostate gland. Organ weight changes were also noted in high dose animals. Scattered deposits of haemosiderin pigment in the liver were observed in the highest dose groups. Bile duct proliferation and eosinophilic degeneration of hepatocytes was also noted at 8000 ppm. Foci of basophilic hepatocytes were also noted for a few animals at the highest doses.

Mice

Mice, 28-day, oral (diet) (Hunter et al., 1979)

Mice (CD-1) were exposed to isoproturon at dose levels of 0, 80/4000 (increased after 2 weeks), 400 and 2000 ppm. 2000 ppm corresponds to 307/378 mg/kg bw/d in males/females. Feed consumption was unaffected by treatment. Mice receiving 4000 ppm for the second half of the study showed slightly reduced body weight gain (significant in males only). Organ weight analysis revealed a slight increase in liver weight when adjusted for body weight in animals receiving 4000 ppm and 2000 ppm.

Dog

Dogs, 28-day, oral (diet) (Kramer and Brunk, 1975)

Dogs (Beagle) were exposed to isoproturon at dose levels of 0, 50, 160 and 500/1250 (increased after 15 days) ppm in the diet. 50 ppm corresponded to 3.3 mg/kg bw/d. Of the 4 dogs receiving 500/1250 ppm one female showed generalised icterus, strongly exsiccotic skin, very poor state of nutrition and distinctly impaired general condition. Two females vomited repeatedly during the first week after the increase in the dose level, mucous membranes were noted to be icteric in one bitch and were found to be pale in the other. At 500/1250 ppm the weight of the animals fell more markedly after the dose increased to 1250 ppm (mean body weight loss for the experiment: -2.5 kg). During the second half of the trial top dose animals showed a reduction of feed intake ranging from 26.2 to 72.8%. An exception was one dog whose feed intake had already fallen by 48.6% during the first half of the trial. At 500/1250 ppm signs of haemoconcentration were observed in the icteric bitch and signs of anaemia (decreased haemoglobin concentration) in the other bitch. Pronounced deposition of haemosiderin in the reticular cells with increased erythrophagocytosis was observed in the two highest dose groups. Some dogs in the high dose group showed reduction of erythrocytic and granulocytic precursors.

Dogs, 90-day, oral (diet) (Scholz and Brunk, 1973)

Dogs (Beagle) were exposed to isoproturon at dose levels of 0, 50, 160 or 500/800 ppm (increased after 15 days) over a 90-day period. 50 ppm corresponded to 3.8 mg/kg bw/d. Dogs in the high dose group showed vomiting after dose increase. At 160 ppm three dogs showed pale mucosae from week 4, 6 and 10, respectively, onwards. Decreased body weight were shown in the high dose group, particularly after the increase of dose (mean body weight loss during the study: -3.4 kg). Feed consumption in these animals was markedly decreased after the dose increase (ranging from 16.8 to 66.1%). In the high dose group 6/8 dogs revealed toxic haemolytic anaemia with concomitant formation of Heinz bodies in 4 cases. Hb was decreased by 21% in males and 20% females at 500/800 ppm. Five out of 8 animals showed impaired nutritional state and small prostate glands were observed in 2 dogs. Liver weights were dose-dependently increased in the two highest dose groups. Dogs receiving 160 or 500/800 ppm showed deposition of haemosiderin in reticular cells, increased erythrophagocytosis of the bone marrow and moderate or moderately pronounced haemosiderosis of Kupffer cells of the liver; all those elements reflecting the form of haemolytic anaemia.

Dogs, 90-day, oral (diet) (Bhide, 1990)

Dogs (Beagle) were exposed to isoproturon at dose levels of 0, 50, 150 or 500 ppm in the diet over a 90-day period. 50 ppm corresponded to approximately 3.8 mg/kg bw/d. In addition a group was treated with 500 ppm for 90 days and observed for reversibility of toxic effects for a post-treatment period of 28 days. Dogs in the high dose group showed a decrease in body weight gain (10-16%) and feed consumption (17-23%), however they were found to regain the body weight gain after termination of the treatment. Emesis, intermittent diarrhoea and reduced locomotor activity were seen at this dose group. A slight reduction in body weight gain was observed in animals treated with 150 ppm, and one male exhibited emesis and diarrhoea. Hb was decreased by 16,2% in males and 20% females at 500 ppm. Methaemoglobin was decreased by 20% in females at 50 and 150 ppm and increased by 400% in males and 337% in females at 500 ppm. PCV and RBC were decreased and Heinz bodies was seen in animals of the high dose group. Total protein in blood were slightly decreased. Haemosiderosis (1 animal), lymphoid depletion (1 animal) and hyperplasia of bone marrow (1 animal) were also observed in this group.

Monkey

Monkeys, 90-day, oral, (gavage) (Bhide, 1984)

Monkeys were exposed to isoproturon at dose levels of 0, 50, 150 and 450 mg/kg bw/d. One group was exposed to isoproturon at a dose of 150 mg/kg bw/d for 90 days and observed for reversibility of toxic effects for a post-treatment period of 30 days. Animals in the two highest dose group exhibited a decrease in locomotor activity and diarrhoea. Haemorrhagic patches on the upper eyelids and gradual cachexia was also seen in the high dose group and one monkey in this group died on the 74th day of treatment. A reduction in body weight gain and a slight reduction in the feed intake was observed in animals in the two highest dose groups. Hb was reduced by 21.4% in males and 18.2% in females at 150 mg/kg bw/day and 56% in males and 57% in females at 450 mg/kg bw/d. A reduction in PCV and RBC was noted in both male and female animals treated with doses of 150 mg/kg bw/d. This reduction was marked at 450 mg/kg bw/d. Animals in the highest dose group also showed an increase in reticulocyte count and methaemoglobin and Heinz bodies were observed. The relative weight of the spleen was elevated in the two highest dose groups.

Repeated dose toxicity: dermal

21-day dermal subacute study in albino rats (no guideline, not GLP) (Dikshith, 1982)

Rats were exposed to isoproturon at dose levels 0, 250, 500 or 1000 mg/kg bw/d for 3 weeks. There were 2 mortalities, one in each of the groups receiving 1000 and 500 mg/kg bw/d. A decrease in body weight was noted in females at all 3 dose levels. In males, no clear dose-related effects on body weight development were observed. At 1000 mg/kg bw/d there was a reduction in feed consumption, at 500 mg/kg bw/d the reduction in feed consumption were only seen in males. RBC counts were decreased in males at all dose levels but in females only at the high dose level. Hb, neutrophil and were decreased at all dose levels while lymphocytes were increased.

Acute dermal toxicity study in rabbits (no guideline, not GLP, considered supplementary) (Hollander and Weigand, 1975)

Rabbits were exposed to isoproturon at dose levels 400 or 800 mg/kg bw/d applied onto the depilated nape skin for 5 successive days. One mortality occurred in the 800 mg/kg bw/d dose group (3 days after the end of treatment). Body weights decreased in rabbits of both treatment groups over the treatment period. By end of the 17-day observation period all surviving animals had regained or exceeded their initial weight.

21-day subacute dermal toxicity study in rabbits (no guideline, GLP compliant) (Bhide, 1996)

Rabbits were exposed to isoproturon at dose levels 0, 500, 1000 or 2000 mg/kg bw/d, applied 5 days per week for 6 h/day for 3 consecutive weeks. No relevant findings was recorded at any dose level.

Percutaneous 90-day toxicity study in rabbits (similar to OECD TG411, GLP) (Bhide, 1990*)*

Rabbits were exposed to isoproturon at dose levels 0 and 1000 mg/kg bw/d, applied 5 days per week for 6 h/day for 13 consecutive weeks. No observable effects was recorded.

Repeated dose toxicity: inhalation

2-week inhalation toxicity study in Sprague Dawley rats (Owen and Glaister, 1982)

Rats were exposed to isoproturon at the target concentrations of 0, 10, 50 or 250 mg/m^3 of a respirable dust, whole body, 6 h/day, 5 days/week for 2 weeks. Three animals that died during the routine orbital sinus bleed procedure had reddened lung lobes. One of these animals (a control) had reddened liver lobes. Males receiving 250 mg/m^3 showed increases in absolute and relative (to body weight) mean liver weight. A low grade interstitial pneumonitis was present in

most animals but the incidence and severity of this finding was similar for the treated and control animals.

Subacute inhalation toxicity study in albino rats (14 days nose only inhalation exposure) (Anonymous, 1985)

Rats were exposed to isoproturon at the concentrations of 0, 173.61 and 664.49 mg/m^3, 6 h/day, 5 days/week, nose only exposure, for 14 days. Mean body weights of all groups were depressed during the first week of exposure and continued to decrease throughout the rest of the exposure period in the high dose group. The lung tissues of some animals from the high dose group revealed interstitial pneumonitis with mononuclear cell infiltration.

90-day inhalation toxicity, Wistar rats (Bhide, 1990)

Rats were exposed to isoproturon at concentrations of respirable dust of 0 and 6.32 mg/L, 6 h/day, 5 days/week for 13 weeks. Respiratory irritation was observed at the high dose at the end of the exposure period.

The key findings from the subacute and subchronic studies with isoproturon, performed in rats, dogs, mice and monkeys by oral administration are summarised in the table below:

Table: Summary table of relevant repeated dose toxicity studies, oral exposure

Study	**Doses relevant for STOT RE 1**	**Effects at this dose level**	**Doses relevant for STOT RE 2**	**Effects at this dose level**
30-day dietary Rat 0-500-1250-3200-8000-20000 ppm Similar to OECD TG 407, not GLP Scholz and Weigand, 1973	Cat 1: C ≤ 30 mg/kg bw/d	N/A	Cat 2: 30 < C ≤ 300 mg/kg bw/d	Reduced body weight gain seen at 500 ppm (43 mg/kg bw/d) Increased relative liver weight (significant) from 1250 ppm (105 mg/kg bw/d) in females and from 3200 ppm (259 mg/kg bw/d) in males.
4-week dietary Mouse 0-80/4000-400-2000 ppm Similar to OECD TG 407, not GLP Hunter *et al*., 1979	Cat 1: C ≤ 30 mg/kg bw/d	N/A	Cat 2: 30 < C ≤ 300 mg/kg bw/d	None. Slightly increased liver weight at 2000 and 4000 ppm when adjusted for body weight, however the effects was only significant in males, and only when adjusted for body weight. 2000 ppm corresponded to 307/378 mg/kg bw/d in males/females
4-week dietary Dog 0-50-160-500/1250 ppm No test guideline, not GLP Kramer and Brunk 1975	Cat 1: C ≤ 30 mg/kg bw/d	Reduced body weight gain, effects on liver, bone marrow and hematological parameters starting at 160 ppm (≈10,5 mg/kg bw/d)	Cat 2: 30 < C ≤ 300 mg/kg bw/d	
13-week dietary Rat 0-80-400-2000-10000→20000 ppm Similar to OECD TG 408, not GLP Leuschner *et al*., 1973	Cat 1: C ≤ 10 mg/kg bw/d	None.	Cat 2: 10 < C ≤ 100 mg/kg bw/d	None.
13-week gavage Rat 0-85-250-750 mg/kg bw/d No test guideline, not GLP	Cat 1: C ≤ 10 mg/kg bw/d	N/A	Cat 2: 10 < C ≤ 100 mg/kg bw/d	None.

Bhide, 1984				
13-week dietary Rat 0-400-1500-5000 ppm Similar to OECD TG 408, not GLP Dickhaus and Heisler 1987	Cat 1: C ≤ 10 mg/kg bw/d	N/A	Cat 2: 10 < C ≤ 100 mg/kg bw/d	Reduced body weight, liver weight increased seen from 1500 ppm (75-150 mg/kg bw/d).
13-week dietary Rat 0-400-1200-2400 ppm Similar to OECD TG 408, GLP claimed Bhide, 1990	Cat 1: C ≤ 10 mg/kg bw/d	N/A	Cat 2: 10 < C ≤ 100 mg/kg bw/d	Slightly reduced body weight gain, haemosiderosis, hyperplasia of bone marrow cells starting at 1200 ppm (60-120 mg/kg bw/d)
13-week dietary Rat 0-80-800-8000 ppm Similar to OECD TG 408, GLP claimed Wragg *et al*., 1991	Cat 1: C ≤ 10 mg/kg bw/d	None.	Cat 2: 10 < C ≤ 100 mg/kg bw/d	Reduced feed intake and body weight gain, liver, reduced RBC, increased methaemoglobin, starting at 800 ppm (62 mg/kg bw/d)
13-week dietary Dog 0-50-160-500→800 ppm Similar to OECD TG 409, not GLP Scholz and Brunk 1973	Cat 1: C ≤ 10 mg/kg bw/d	None.	Cat 2: 10 < C ≤ 100 mg/kg bw/d	Increased liver weight, Hb decreased (approx. 20% at 500/800 ppm), haemolytic anemia 500/800 ppm corresponded to approximately 38-61 mg/kg bw/d
13-week dietary Dog 0-50-150-500 ppm Similar to OECD TG 409, GLP Bhide, 1990	Cat 1: C ≤ 10 mg/kg bw/d	None.	Cat 2: 10 < C ≤ 100 mg/kg bw/d	Reduced feed intake and body weight, Hb reduced by 20% in females at 500 ppm. MetHb increased with 337-400% at 500 ppm (38 mg/kg bw/d).
13-week gavage Monkey 0-50-150-450 mg/kg bw/d No test guideline, not GLP Bhide, 1984	Cat 1: C ≤ 10 mg/kg bw/d	N/A	Cat 2: 10 < C ≤ 100 mg/kg bw/d	None.

N/A = Not applicable (no relevant dose in the study).

The criteria for classification with STOT RE 2 is considered to be fulfilled for haematological effects occurring in the oral 90-day repeated exposure studies dogs. According to the Guidance on the application of the CLP criteria, section 3.9.2.5.2 a substance could be classified for STOT RE e.g. based on a reduction of Hb ≥ 20%. The 90-day studies in dogs showed haematotoxicity (decrease in Hb by 20 % along with haemosiderin deposition in reticular cells and Kupffer cells of the liver), at doses between the guidance values for classification with category 2 of 10 mg/kg bw/d and 100 mg/kg bw/d.

The presented repeated dose toxicity studies by inhalation and dermal routes show no haematoxicity relevant for classification However, there are no studies by inhalation and dermal routes in dogs which are shown to be the most sensitive species by the oral route. It can therefore not be disregarded that similar effects will occur also from these routes of exposure, and therefore no route is proposed to be specified.

Overall, RAC concludes that the DS proposal to classify isoproturon as **STOT RE 2, H373**: "May cause damage to organs (blood) through prolonged or repeated exposure" is justified without specifying a route of exposure.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify isoproturon with Repr. 2, H361f on the basis of evidence of reduced male fertility in rats. In the two-generation reproduction toxicity studies histopathological changes in the testes revealed retarded spermatogenesis. The results of a supplementary published study confirmed an affected spermatogenesis in rats possibly based upon impaired androgen biosynthesis at high doses. Reproductive toxicity was observed at clear parental toxicity. However, the DS stated that there was no clear evidence to conclude that the observed reproductive toxicity was solely produced as a non-specific secondary consequence of parental toxicity.

Fertility

Four 2-generation studies in rats were described by the DS. In one two-generation reproduction toxicity study in rats the mean number of implantation sites and the corresponding number of living pups per litter were statistically significant decreased at a dietary dose level of 2000 ppm (134-263 mg/kg bw/d). Feed consumption and body weights of the F0 and F1 generations were statistically significant reduced at this dose level during all periods.

Three other two-generation studies in rats were all performed by the same laboratory, under similar conditions, and using dose levels up to 400 ppm. These studies demonstrated a lower pregnancy rate and a lower mating index of F1 females at 400 ppm (40 mg/kg bw/d), histopathological changes in the testes (retarded spermatogenesis) of F1 animals at 200 ppm (20 mg/kg bw/d) and above or at 400 ppm (40 mg/kg bw/d). Parental toxicity (reduced feed consumption and body weight gain) was seen in the studies at dose levels of 400 ppm (40 mg/kg bw/d). Histopathological changes in the liver and the spleen of F1 animals were seen from 200 ppm (20 mg/kg bw/d).

Further, one supplementary study on male reproductive system is described showing decreased epididymal sperm counts and motility, and increased percentage of abnormal sperm in rats treated with 800 mg/kg bw/d for 6 days/week for 10 weeks. Histological changes were seen at 400 mg/kg bw/d and above.

Development

Seven teratogenicity studies are presented by the DS. In the rat developmental toxicity studies using dose levels up to 500 mg/kg bw/d there was no evidence of teratogenicity. Maternal toxicity (reduced body weight gain and feed consumption) and embryo/foetotoxicity (increase in resorptions, reduced foetal weight, incomplete ossification) were seen at 100 mg/kg bw/d and above.

There was no evidence of teratogenicity in two rabbit developmental toxicity studies using dose levels up to 160 mg/kg bw/d. Maternal toxicity (reduced body weight gain and feed consumption) was seen from 100 mg/kg bw/d and embryo/foetotoxicity (reduced foetal weight) was seen at 160 mg/kg bw/d.

Comments received during public consultation

Four MSCAs supported the proposed classification with Repr. 2, H361f. However, some of the MSCAs pointed out that there is a lack of data in the CLH report and partly also in the DAR. One MSCA was supportive of the proposed classification, but pointed out that to justify classification, data should be reported and discussed more accurately. One MSCA also commented that it is not quite clear why category 2 is chosen instead of category 1B. In addition, one MSCA was of the opinion that some effects on development (increased resorptions) should be taken into consideration.

Three position papers from Industry argued for no classification for reproductive toxicity. They were of the opinion that the findings on retarded spermatogenesis is not sufficient to justify classification, since the retarded spermatogenesis seen in 3 two-generation studies from the same laboratory are not seen in a two-generation study from another laboratory. In addition they commented that the retarded spermatogenesis is not appropriately described, that there may be flaws in the fixation during tissue sampling or during staining, and pointed out that none of the sub-chronic and chronic/carcinogenicity (Hunter *et al*., 1981; not reported in the CLH report) toxicity studies describe effects on testes. They also questioned the unusual rat strain used in the 3 two-generation studies from the same laboratory (Wistar animal colony "IIT Animal House") and their extremely low body weight at the start of dosing and during the study (approximately one third the body weight of Wistar rats used in Becker, 1989). The DS responded that considering the fact that effects are only observed at dose levels also inducing parental toxicity, and the fact that effects are not consistently observed in all generations, there is only some evidence for effects on fertility (Repr. 2) as also concluded by EFSA.

The supplementary 10-week study by Sarkar *et al*. (1997) was considered by Industry as unreliable due to numerous flaws in the experimental design and reporting. The doses were 10-fold higher than in the two-generation studies and there is no reporting of systemic toxicity of the treatment, which would be expected due to effect seen in the two-generation studies. Further the body weight of the rats were questioned as well as the number of animals examined and the microscopy methods used. The DS was of the opinion that the supplementary published study showed some evidence of an affected spermatogenesis in rats possibly based upon impaired androgen biosynthesis at high doses.

Assessment and comparison with the classification criteria

Four 2-generation studies in rats and seven teratogenicity studies (rats and rabbits) are presented by the DS. The table below summarises the details of these studies as they are presented in the DAR.

T*able: summary of reproductive toxicity studies conducted with isoproturon*

Study	**Dose levels**	**Target/main effects**
2-generation, Wistar/HAN rat OECD TG 416 (deviations; F0 males only dosed 56 days prior to pairing) GLP-compliant Study considered acceptable. Becker *et al*., 1989	0-80-400-2000 ppm, groups of 25/sex, oral exposure via diet (Doses corresponding to daily intake ranging from 0-275 mg/kg bw/d)	Feed intake, bw, implantations, litter size, pup weight ↓ No deaths occurred in the animals of either generation. No treatment-related signs of toxicity or clinical symptoms were evident. Feed consumption was statistically significantly reduced in the 2000 ppm group of the F0 and F1 generations during all periods. At 2000 ppm both F0 and F1 generations showed that the mean number of implantations sites and the corresponding number of living pups per litter were slightly but statistically significant reduced (9% reduction in implantations sites and 5-16% reduction in living pups). The mean body weights of pups were statistically significant reduced at 2000 ppm on day 1 post-partum and throughout the lactation period (reduction of approximately 7% at day 1, and 28% at day

		21). At 400 ppm, moderately but statistically significant decreases in the mean body weight weight were noted from day 14-21 post-partum for the F1 pups and from day 7-21 postpartum for the F2 pups.
2-generation, Wistar rat, OECD TG 416 (deviations; number of females successfully mated and pregnant not reported, presence of vaginal sperm confirmed only in some females, pup survival rate from birth to day 4 not reported) GLP-compliant Study considered supplementary. Bhide *et al*., 1990	0-100-200-400 ppm, groups of 15 males/30 females, oral exposure via diet	Bw; pregnancy rate, pup weight ↓, retarded spermatogenesis No deaths occurred in the animals of either generation. No treatment-related signs of toxicity or clinical symptoms were evident. F1 males and females in the highest dose group had signigicantly reduced body weight. The pregnancy rate was affected in the F1 females in the higest dose group. Retarded spermatogenesis and focal hyperplasia in seminal vesicles and prostate were observed in 2, 1 and 1 out of 15 animals, respectively, in the F1 generation at 400 ppm. Retarded spermatogenesis was also noted in one out of 15 animals at 200 ppm. Hydropic degeneration in liver and lymphoid hyperplasia in spleen were seen in some male and female animals from intermediate and high dose groups.
2-generation, Wistar rat, OECD TG 416 GLP-compliant Study considered acceptable. Bhide, 1991	0-100-200-400 ppm, groups of 15 males/30 females, oral exposure via diet	Feed intake, bw; mating index, pup weight ↓, retarded spermatogenesis No deaths occurred in the animals of either generation. No treatment-related signs of toxicity or clinical symptoms were evident. F1 males and females in the highest dose group had statistically significant reduced body weight (23-24%) and feed consumption (8%). The mating index was reduced in females in the F1 generation receiving the high dose. There were no effects on litter size, sex ration, number of live and dead pups and survival rate of the pups. F2 pups in the high dose group had reduced body weight gain compared to the controls (26%). Retarded spermatogenesis were observed in 3 out of 15 parental animals (20%) at 400 ppm and one out of 15 parental animals at 200 ppm in F1. Focal hyperplasia in seminal vesicles and prostate were observed sporadically at 200 and 100 ppm. Hydropic degeneration in liver and lymphoid hyperplasia in spleen were seen at 400 ppm, an also sporadically at 200 and 100 ppm.
2-generation, Wistar rat OECD TG 416 (deviations; pup survival rate from birth to day 4 not reported) GLP-compliant Study considered supplementary. Bhide, 1991	0-100-200-400 ppm, groups of 15 males/30 females, oral exposure via diet	Feed intake, bw; pregnancy rate, pup weight ↓, retarded spermatogenesis No deaths occurred in the animals of either generation. No treatment-related signs of toxicity or clinical symptoms were evident. F1 males and females in the highest dose group had statistically significant reduced body weight and feed consumption (17-28% and 10%). The pregnancy rate was reduced in females in the highest dose group in the F1 generation. There were no effects on litter size, sex ratio, number of live and dead pups and survival rate of the pups. F2 pups in the high dose group had reduced body weight gain compared to the controls (22%). Retarded spermatogenesis were observed in the F1 generation in 2 out pf 15 animals at 400 ppm and 2 ouf of 15 animals at 200 ppm. Focal hyperplasia in seminal vesicles and prostate were observed in 1 out of 15 animals at 200 ppm. Hydropic degeneration in liver was seen at 400 ppm (1 out of 15 males/2 out of 30 females) and lymphoid hyperplasia in spleen were seen at 400 ppm, an also sporadically at 200 and 100 ppm.
10 weeks, effect of IPU on male reproductive system, male albino rats	0, 200, 400 or 800 mg/kg bw/d, 6 days/week	Epididymal sperm counts and motility ↓, abnormal sperm ↑, damaged seminiferous tubules ↑, impaired formezan deposition from glucose-6-phosphate and ß-hydroxysteroid dehydrogenase Decreased epididymal sperm counts (75%) and motility (56%), and increased percentage of abnormal sperm were observed at 800

Study considered supplementary. No guideline, not GLP Sarkar *et al*., 1997		mg/kg bw/d in rats treated 6 days/week for 10 weeks (increased from 4% in control to 30% at highest dose). Histological changes of the testes and histochemical activity of selected enzymes in testicular tissue were seen at 400 mg/kg bw/d and above. There is no information on treatment-related signs of toxicity or clinical symptoms in the rats included in the publication.

Fertility

In the two-generation reproduction toxicity study in rats using dietary dose levels up to 2000 ppm, parental toxicity (reduced body weight gain and feed consumption) was seen at dose levels of 400 ppm and above. For both F0 and F1 generations, the mean number of implantations, litter size and pup weight were slightly but statistically significantly decreased at doses of 2000 ppm.

Three other two-generation studies where all performed by the same laboratory, using the same dose levels up to 400 ppm. These studies all showed evidence of retarded spermatogenesis in F1 males at doses of 200 ppm and above, and the findings were consistent across all three studies. At 400 ppm retarded spermatogenesis was reported in 13-20% of the males. A decrease in either mating index or pregnancy rate was also observed in these studies at 400 ppm. Parental toxicity (decreased body weight) was only visible at 400 ppm. It is noted that all effects are only observed in F1 animals breeding for F2, and not in F0 animals breeding for F1.

It should be noted that there are considerable differences between the body weights of the rats in the different 2-generation studies. In the three studies by Bhide *et al*. the rats have an initial weight of approximately 60 grams, while in the study by Becker *et al*. the rats have an initial weight of approximately 220(m)/170(f) grams. However, the body weight gain during the study appears to be comparable in all four studies. There are a statistically significant reductions in body weight in the F1 animals exposed to 400 ppm when compared with the control group in all four studies. In addition the body weight is statistically significant reduced in both the F0 and F1 generation exposed to 2000 ppm compared to the control group in the Becker study. The different body weights are presented in the table below:

Table: comparison of body weight changes in reproductive toxicity studies

Study		0 ppm m/f	100 ppm m/f	200 ppm m/f	400 ppm m/f
Bhide *et al*., 1990	F0, bw, wk 0 (g)	58.4/58.9	58.4/59.3	61.8/59.1	60.0/60.2
	F0, bw, wk 13 or 10 (g)	189.3/158.6	196.1/158.9	189.0/156.0	187.5/158.1
	F1, bw, wk 0 (g)	33.4/35.8	35.4/35.5	35.7/35.9	35.1/35.8
	F1, bw, wk 12 (g)	164.7/145.4	162.1/141.6	159.3/141.3	**120.6/118.7***
Bhide *et al*., 1991	F0, bw, wk 0 (g)	62.0/60.4	61.3/60.6	61.6/59.6	61.1/59.9
	F0, bw, wk 13 or 10 (g)	226.6/174.4	224.9/171.3	225.1/168.1	217.5/166.8
	F1, bw, wk 0 (g)	34.3/34.7	34.9/34.8	34.9/35.3	34.1/34.9
	F1, bw, wk 15 or 12 (g)	217.2/167.3	218.2/166.1	215.6/163.1	**167.2/126.1***
Bhide *et al*., 1991	F0, bw, wk 0 (g)	61.1/61.2	61.7/61.4	60.7/60.3	60.3/59.8
	F0, bw, wk 13 or 10 (g)	222.5/165.9	227.3/167.2	225.9/162.1	220.2/162.8
	F1, bw, wk 0 (g)	34.5/34.4	34.0/34.1	34.6/34.7	33.9/33.7
	F1, bw, wk 15 or 12 (g)	193.9/141.2	186.6/142.1	177.9/140.1	**138.8/117.7***

Study		0 ppm m/f	80 ppm m/f	400 ppm m/f	2000 ppm m/f
Becker *et al*., 1989	F0, bw, day 1 (g)	219/173	220/171	220/173	217/173
	F0, bw, day 56 (g)	381/236	383/233	379/229	**338/209***
	F1, bw, day 1 (g)	143/121	138/116	138/116	**99/92***
	F1, bw, day 124 (g)	485/275	477/272	**452/257***	**390/220***

*Statistically significant different from control
M=males; f=females

A non-guideline, non-GLP supplementary 10-week study in rats showed effects on spermatogenesis, which could imply an impaired androgen biosynthesis. Histological changes were seen at 400 mg/kg bw/d and above. The doses used in this study was 10-fold higher than the doses used in the two-generation studies, however they are comparable to the doses used in the repeated dose studies. There were no reporting of toxic effect seen in the treated animals. Such effects could have been expected considering effects seen in other studies using lower doses.

It should also be taken into account that very limited effects on testes histopathology were reported in the available repeated dose toxicity studies. The repeated dose toxicity study by Wragg *et al*. (1991) may indicate effects on male fertility as small seminal vesicles and a small prostate gland were seen in 6/10 male rats exposed to 8000 ppm isoproturon in the diet for 90 days. Taken together, these studies show some evidences for effects of isoproturon on toxicity to fertility. However since there are methodological and reporting deficiencies in several of these studies, the effects are not fully convincing. In particular, the reporting of retarded spermatogenesis is questioned, due to insufficient description of the specific parameters measured and the effects seen.

In the evaluation of the parental toxicity seen in the 2-generation studies, it could be relevant also to take the effects seen in the repeated dose studies into consideration. The dog were the most sensitive species for the haematotoxicity seen in these studies. In the three different 90-day studies in rats effects were seen starting at 800, 1200 and 1500 ppm, respectively. NOAELs for these three studies are reported to be 80, 400 and 400 ppm, respectively. Findings included e.g. reduced feed intake and body weight gain, increased liver weight, and effect on various blood parameters. However, these effects would not be sufficiently adverse to be considered as marked systemic toxicity. RAC agrees that the effects on fertility occurred together with other toxic effects but it is questionable wether the effects can be considered a secondary non-specific consequence of the other toxic effects or not.

RAC therefore concludes that classification for effects on fertility is not warranted. As there is no human evidence to suggest that isoproturon is a known human reproductive toxicant, category 1A is not appropriate. In consideration of category 1B and 2, it is noted that the reporting of the effects seen are insufficient. The effects were not pronounced and were seen at doses also showing some parental toxicity (reduced body weight gains and feed consumption). Considering this, RAC agrees with the DS that the strength of evidence appears too weak to require a classification.

RAC therefore agrees with the DS that **no classification of isoproturon for effects on fertility** is justified.

Development:

Study	**Dose levels**	**Target/main effects**
Teratogenicity, CD rat OECD TG 414 (some deviations), not GLP, Supplementary Fritz, 1978	0-25-100-200 mg/kg bw/d, intragastric intubation during GD 6-15, 25 mated females/group	Bw, feed intake ↓; retarded ossification Body weight and feed consuption were slightly reduced at 100 mg/kg bw/d and markedly reduced at 200 mg/kg bw/d. Deciduomata were found in 2 out of 25 females at 200 mg/kg bw/d. Slight delay of skeletal development (increase in the number of unossified calcanei (100 and 200 mg/kg bw/d) and unossified phalangeal nuclei of the forelimb (200 mg/kg bw/d)).
Teratogenicity, Wistar rat OECD TG 414 (deviations), not GLP Not acceptable Sengupta, 1985	0-90-500 mg/kg bw/d, details on test subatance administration is missing	Mortality ↑, bw ↓ at 500 mg/kg bw/d. No evidence of embro/fetotoxicity/teratogenicity.
Teratogenicity, SFP Wistar rat OECD TG 414 (some deviations), not GLP Acceptable Dickhaus and Heisler, 1987	0-20-80-320 mg/kg bw/d, intragastric intubation during GD 6-15, 20 mated females/group	Bw gain ↓, foetal weight ↓(, resorption index ↑ No sign of maternal toxicity, no mortalities. Body weight gain decreased (statistically significant in the high dose group). Increased number of resorptions (high dose group) There were no effects on number of implantations, litter size or foetal malformations and variants. Foetal weights and placenta weights were decreased (statistically significant in the high dose group)
Teratogenicity, Wistar rat OECD TG 414 (some deviations), not GLP Supplementary Katdare *et al*., 1991	0-125-250-500 mg/kg bw/d intragastric intubation during GD 6-15, 20 mated females/group	Clinical signs, feed intake ↑; resorption index ↑ Signs of maternal toxicity at 500 mg/kg bw/d (excessive urination or lethargy from GD 18-20). No mortalities. Feed consumption was significantly increased GD 6-20. No dose related effect on body weight gains, body weights were unusually higher in all dosage groups as compared to controls. Resorptions was increased at 250 mg/kg bw/d and 500 mg/kg bw/d (statistically significant at highest dose), however at the highest dose the females showed abnormal clinical signs including execcive urination and lethargy. There were no effects on numbers of implantations, litter size, foetal malformations and variants or foetal body weight.
Teratogenicity, Wistar rat Supplementary Srivastava & Raizada, 1995	0-45-90-180 mg/kg bw/d,	No developmental effects No further details on this study are provided.
Teratogenicity, Chinchilla rabbit OECD TG 414 (some deviations), not GLP Acceptable Fritz *et al*., 1978	0-12.5-50-100 mg/kg bw/d oral gavage during GD 6-18, 20 mated females/group	Bw, feed intake ↓ Maternal toxicity (decreases in body weight gain and feed consumption) evident in the 100 mg/kg bw/d group. No mortalities. There were no effects on pregnancy rates, number of implantations, number of resorptions, litter size, sex ratio or foetal malformations and variants. The few observed instances of malformations were considered to be spontaneous origin. Average weight of female foetuses from the 50 mg/kg bw/d dose group was statistically significant reduced (not considered to be treatment related).
Teratogenicity, NZW rabbit OECD TG 414, not GLP Acceptable Dickhaus and Heisler, 1987	0-10-40-160 mg/kg bw/d oral gavage during GD 6-18, 12 mated females/group	Bw, feed intake, foetal weight ↓ No clinical signs of maternal toxicity. No mortalities. Body weight gain and feed consumption were statistically significant decreased at 160 mg/kg bw/d. No effects on numbers of implantations, resorptions, litter size or foetal malformations and variants. Foetal weights and placenta weights statistically significant decreased and foetuses with weights lower than 70% of the mean litter weight was clearly increased at 160 mg/kg bw/d.

The available teratology studies conducted in rats and rabbits do not provide any findings to justify classification of isoproturon for developmental toxicity. Maternal toxicity (reduced body weight gain and feed consumption) were seen at doses of 100 mg/kg bw/d and above. Embryo/foetotoxicity (increase in resorptions, reduced foetal weight, and incomplete ossification) were seen from 100 mg/kg bw/d (rat) and 160 mg/kg bw/d (rabbit). The observation of retarded ossification (statistically significant at the highest dose) was only seen in one supplementary study (rat). Another study in rats showed a dose-related increase in resorptions (statistically significant). However, at this dose the females also showed abnormal clinical signs as excessive urination and lethargy. Two other studies showed reduced foetal weight, but only at doses also showing reduced maternal weight.

RAC therefore agrees with the DS that **no classification for development** is justified.

Based on the presented data, RAC concludes that **no classification is warranted for reproductive toxicity of isoproturon**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier submitter's proposal

Isoproturon has currently a harmonised classification as Aquatic Acute 1 and Aquatic Chronic 1, with an M-factor of 10 for both aquatic hazards, according to the CLP Regulation.

Isoproturon was added to Annex I of Directive 67/548/EEC with the 25th ATP (Commission Directive 1998/98/EC of 15 December). The main argument for the environmental classification was the lack of data on biodegradation and aquatic toxicity.

The DS proposed to retain the current environmental classification for isoproturon as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410). The classification was based on the substance being not rapidly degradable, non-bioaccumulative and very toxic to algae and aquatic plants. The relevant lowest acute aquatic toxicity value was a 72h-ErC_{50} of 0.046 mg/L on algae (*Navicula pelliculosa)* and the lowest chronic aquatic toxicity values were a 14d-NOErC of 0.0019 mg/L on aquatic plants (*Lemna gibba*) and a 72h-NOErC of 0.0064 mg/L on algae (*Navicula pelliculosa*).

Degradation

The substance was found to be stable by direct aqueous photolysis (DT_{50} of 72-88 days). None of the four degradation products were identified in concentrations of more than 5.4% of the applied radioactivity.

The radiolabelled substance was found to be hydrolytically stable for up to 30 days at pH 4, 5, 7 and 9 at 25º and 50ºC after a test carried out according to EEC method C.7. Seven hydrolysis products were detected but the major metabolite, 4-isopropylaniline, did not exceed 10% of the applied radioactivity at 25ºC and ranged 18-62% at 50ºC after 30 days.

Regarding biodegradability, one study on ready biodegradability and four simulation studies on biodegradation in water/sediment systems are available. In addition, the results of three of the simulation studies have been re-calculated based on the original raw data and newly submitted studies according to FOCUS Degradation Kinetics (2006).

The study on ready biodegradability was performed according to OECD TG 301B (Modified Sturm test) and GLP principles. The mean biodegradation of isoproturon after 28 days was 3% and

therefore the substance was considered not readily biodegradable under the test conditions. Isoproturon showed no inhibitory effects on the activated sludge micro-organisms at the tested concentrations.

In the water/sediment simulation studies half-lives were estimated for the whole system and for water, while it was not possible to determine the rate of degradation in sediment. The half-lives from the original studies and the re-calculated half-lives showed that the degradation in water/sediment systems is slow; DT_{50} values for the whole system ranged from 50.7 to 299.6 days with a geometric mean of 129.3 days (DT_{90} from 168.5 to 995.4), and DT_{50} values for water ranged from 27.2 – 198.9 days at 20°C with a geometric mean of 61 days (DT_{90} from 90.5 to 284.3, but for one system DT_{90} of water compartment was not available).

Based on the available information, the DS concluded that isoproturon is considered as not rapidly degradable for the purposes of classification and labelling.

Bioaccumulation

Isoproturon has a measured log Kow of 2.6 at 25°C (OPPTS Guideline 830.7570 equivalent to EEC method A.8.). An experimental BCF in rainbow trout (*Oncorhynchus mykiss*) was estimated in a study following OECD TG 305E and GLP principles. Both the uptake and depuration of the substance were rapid. The steady state BCF was estimated to be 2.6 and 3.6 L/kg. Based on the log Kow and the experimental BCF, the DS concluded that isoproturon is not a bioaccumulative substance for classification purposes.

Aquatic Toxicity

Acute and chronic aquatic toxicity data on all three trophic levels (fish, aquatic invertebrates, algae/aquatic plants) are available (Table below). Since isoproturon is a herbicide, algae and aquatic plants were the most sensitive species.

Table: Summary of the relevant toxicity information on fish, aquatic invertebrates and algae/aquatic plants.

Species	Guideline	Test type	Toxicity (mg/L)
Oncorhynchus mykiss	OECD TG 203 (GLP)	static	96h-LC_{50} 23.83 (mm)
Cyprinus carpio	OECD TG 203 (GLP)	static	96h-LC_{50} 41.0 (mm)
Oncorhynchus mykiss	OECD TG 204 (GLP)	semi static	21d-NOEC (growth) 1.0 (nom)
Daphnia magna	EPA 850.1010; FIFRA 72-2 (GLP)	static	48h-EC_{50} 0.58 (mm)
Daphnia magna	OECD TG 211 (GLP)	Semi-static	21d-NOEC 0.12 (nom)
Pseudokirchneriella subcapitata	OECD TG 201 (GLP)	static	72h-EbC_{50} 0.025 (mm) 72h-ErC_{50} 0.098 (mm) 72h-NOEb/rC 0.018 (mm)
Navicula pelliculosa	OECD TG 201 (GLP)	static	72h- EbC_{50} 0.013 (nom) 72h-NOEbC 0.0025 (nom) **72h- ErC_{50} 0.046 (nom)** **72h-NOErC 0.0064 (nom)**
Lemna gibba	US EPA FIFRA Guideline 122-2 and 123-2 (GLP)	Semi-static	14d-EbC_{50} 0.037 (mm) 14d-NOEbC 0.0052 (mm) 14d-ErC_{50} 0.045 (mm) **14d-NOErC 0.0019 (mm)**

mm… mean measured
nom… nominal

Acute (short-term) aquatic toxicity

For fish, two acute studies are available, both of them carried out according to OECD TG 203 and GLP principles. *O. mykiss* was the most sensitive fish species tested in the acute studies, with a 96-h LC_{50} of 23.83 mg/L based on measured concentrations.

One immobilisation toxicity study on *D. magna* conducted according to EPA 850.1010; FIFRA 72-2 is available. The study provided a 48-h EC_{50} of 0.58 mg/L based on mean measured concentrations.

Two toxicity studies on algae (*N. Pelliculosa and P. subcapitata*) conducted according to OECD TG 201 and one study on an aquatic plants (*L. gibba*) following US EPA FIFRA Guidelines 122-2 and 123-2 are available. All studies are in compliance with GLP principles. The most sensitive species for acute toxicity were *N. pelliculosa* with a 72h-ErC_{50} of 0.046 mg/L and *L. gibba* with a 14d-ErC_{50} of 0.045 mg/L (used as supporting information for classification).

Long-term aquatic toxicity

Reliable information on the long-term toxicity of isoproturon to fish is not available. The surrogate approach results in a conclusion of Chronic 1, M-factor = 10. This is supported by a prolonged acute (OECD TG 204) study which does not suggest that a more stringent chronic classification is necessary for fish (based on a 21-d NOEC of 1.0 mg/L for growth inhibition in *O. mykiss*). Since the substance has a low bioaccumulation potential, the classification is unlikely to be affected by a more relevant fish study (e.g. OECD TG 210, 212 or 215).

A chronic reproduction study on *D. magna* is available carried out according to OECD TG 211, and GLP principles, resulting in a 21-day NOEC of 0.12 mg/L based on nominal concentrations. Nominal concentrations were used to calculate NOEC as the chemical analyses performed upon each test solution renewal showed that the measured concentrations were close to the nominal (89-109 % recovery) and the substance was stable in the test solution.

Two toxicity studies on algae conducted according to OECD TG 201 and one study on an aquatic plants following the US EPA FIFRA Guidelines 122-2 and 123-2 are available. All studies are in compliance with GLP principles. The NOECs for biomass and growth rate were determined based on measured test concentrations except for *N. pelliculosa* for which the NOEC was based on the nominal concentrations as the analytical results of the test solutions were within 80 and 120 % of nominal. The most sensitive species for chronic toxicity were *N. pelliculosa* with 72-h NOEbC of 0.0025 mg/L and NOErC of 0.0064 mg/L, respectively, and *L. gibba* with 14-d NOEbC=0.0052 mg/L and NOErC=0.0019 mg/L.

According to the CLH guidance a 7-day growth endpoint for Lemna is preferred to a 14-day endpoint, but no 7-day toxicity data are available in the CLH report. The 14-day NOEC of 0.0019 mg/L for Lemna is the most sensitive end point for classification purposes. Since the study was semi-static, nutrient depletion and pH changes are less likely to have been a problem over 14 days. In addition, loss of test concentration is unlikely since isoproturon shows very limited degradation in both water-sediment systems and abiotic degradation studies, and appears to be stable in other ecotoxicity media at similar pH. Additionally, it is noted that long-term toxicity information for Lemna gibba (14-day) and Navicula pelliculosa (72-h) lead to the same chronic M factor.

Comments received during public consultation

Comments from two MSCAs were received during the public consultation supporting the DS's proposal for environmental classification and M-factors.

Two additional MSCAs also agreed with the proposal for environmental classification and one suggested to add explanations regarding the chronic fish toxicity test and the Lemna gibba test.

Assessment and comparison with the classification criteria

Degradation

Isoproturon is hydrolytically and photolytically stable, is not readily biodegradable (3% degradation) and shows slow biodegradation in water/sediment simulation tests (whole system DT_{50} values of 50.7-299.6 days). Therefore, RAC agrees with the DS's proposal that isoproturon is considered as not rapidly degradable for the purposes of classification and labelling.

Bioaccumulation

RAC agrees with the DS's proposal that isoproturon has a low potential for bioaccumulation based on a measured log Kow of 2.6 and is not expected to bioconcentrate based on an experimental BCF in fish of 2.6-3.6 L/kg (OECD TG 305E).

Aquatic Toxicity

RAC has evaluated the acute and chronic aquatic toxicity data available for all three trophic levels and agrees with the DS's proposal that algae and aquatic plants are the most sensitive organisms, with the observed acute toxicity values in the range of $0.01 < EC_{50} \leq 0.1$ mg/L and chronic toxicity values in the range of $0.001 < NOEC \leq 0.01$ mg/L. The acute toxicity values determined for fish and aquatic invertebrates were in the range of $LC_{50} > 1$ mg/L and $0.1 < EC_{50} \leq 1$ mg/L, respectively, and chronic values in the range of $0.1 < NOEC \leq 1$ mg/L.

The lowest relevant acute toxicity value is a 72-h ErC_{50} of 0.046 mg/L observed for *N. pelliculosa* which is well below the classification threshold of 1 mg/L. The value is in the range of $0.01 < L(E)C_{50} \leq 0.1$ mg/L which justifies an acute M-factor of 10.

The lowest relevant chronic toxicity values are a 14-d NOErC of 0.0019 mg/L determined for *L. gibba* and a 72-h NOErC of 0.0064 mg/L for *N. pelliculosa*. These values are below 0.01 mg/L which is the classification threshold for category Chronic 1 for not rapidly degradable substances, and justify a chronic M-factor of 10 ($0.001 < NOEC \leq 0.01$ mg/L).

Based on the above information, RAC agrees with the DS's proposal that isoproturon fulfils the classification criteria for **Aquatic Acute 1 (H400) with an M-factor of 10 and Aquatic Chronic 1 (H410) with an M-factor of 10**.

Additional references

Additional references not included in the CLH report

Sarkar SN, Chattopadhyay SK, Majudmar AC (1995). Subacute toxicity of urea herbicide, isoproturon, in male rats. *Indian Journal of Experimental Biology* **33**, 851-856.

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 by RAC (excluding confidential information).